UNIVERSITY OF
LINCOLN

Investigating the morphology and functional properties of Sylviidae nests

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13th February 2018

Abstract

Investigating the functional properties of bird nests has received an increased interest in recent years. Despite this, quantitative data for nest composition is scarce for many species and relatively little is known about functional aspects of nest construction across species. This thesis provides additional quantitative data for nest composition and investigates the thermal and hydrological properties of nests from three closely related species from the Sylviidae family; Eurasian Blackcaps *Sylvia atricapilla*, Eurasian Reed Warblers *Acrocephalus scirpaceus* and Willow Warblers *Phylloscopus trochilus*. Willow Warblers built nests with the greatest mass whereas Blackcap nests were the smallest in mass of the three species. Principal component analysis revealed that there was interspecific variation in the composition of warbler nests. Willow Warbler nests were characterised by a larger quantity of moss and feathers than in Reed Warbler and Blackcap nests, with an absence of invertebrate silk and artificial materials. Reed Warbler nests were primarily composed of dry grasses, invertebrate silk and artificial materials. Blackcap nests were constructed with roots and grasses, artificial materials and invertebrate silk. Internal cooling rates were greatest for Blackcap nests under still and moving air conditions in comparison to Reed Warbler and Willow Warbler nests. Internal cooling rates in moving air were the smallest for Reed Warbler nests. Heavier nests with thicker nest floors were better insulating than smaller nests under still air conditions. Warbler nests were able to reduce the effect of air movement on temperature loggers despite the increasing internal cooling rate, suggesting that presence of a nest is beneficial during periods of air movement. Nests with greater grass mass were better at reducing the effect of air movement on the internal microclimate of warbler nests. The effect of test location on the output for thermal data by temperature loggers placed in warbler nests was also investigated by comparing the thermal measures recorded in an open laboratory and an enclosed test chamber. Test location had a significant effect on thermal measurements recorded for nests. Species had a significant effect on the hydrological properties of warbler nests. Willow Warbler nests had the longest drying times and greater water absorption of the three species. Nest materials did not influence the hydrological properties of warbler nests. This thesis provides further quantified data for the

composition of nest materials and builds on the evidence for the relationship between avian nest construction and function.

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Word count: 20872

Chapter 1: An introduction to the construction and function of warbler nests.

Nests are built by almost all avian species and are structures that act primarily as the location for egg laying and chick rearing (Hansell 2000; Heenan 2013). Nest building had previously been thought to be a fixed, innate behaviour but recent evidence suggest that nest building is a plastic and learned behaviour in some species (Collias and Collias 1984; Muth and Healy 2011; Deeming *et al.* 2012; Mainwaring *et al.* 2012, 2014a, 2014b; Bailey *et al*. 2014). Nest building behaviour has been documented for a few species (Healy *et al.* 2015) which describe multiple different methods birds have used for nest construction. Birds may construct nests via the addition or removal of nest materials to or from a nest site (Hansell 1984, 2000; Healy *et al*. 2015). Construction methods, such as weaving, piling, sowing and moulding of nest materials, have previously been reported (Collias and Collias 1954; Hansell 1984, 2000; Healy *et al*. 2015). Interspecific variation in nesting height has previously been reported, for example, different avian species may build nests at different heights above the ground, for example, the Mountain Chickadee *Poecile gambeli* has been reported to nest at 15 metres above ground (Li and Martin 1991) whereas Pectoral Sandpipers *Calidris melanotos* construct scrapes on the ground (Reid *et al.* 2002a). Species such as Blue Tits *Cyanistes caeruleus* build cavity nests inside natural holes or nest-boxes (Deeming *et al.* 2012), while other species such as Eurasian Bullfinches *Pyrrhula pyrrhula* build cup nests in more open environments (Biddle *et al.* 2017).

1.1. Nest construction

Research into nest construction and function in recent years has been accumulating evidence that nests are variable in their morphology within and between species. Previous studies have shown that nests built by individuals of the same species may vary in three-dimensional size, in the type of materials used within the nests and in the structure of the nest (Rowher and Law 2010; Britt and Deeming 2011; Deeming *et al.* 2012; Mainwaring *et al.* 2012, 2014b; Taberner Cerezo and Deeming 2016; Biddle *et al.* 2015, 2017, 2018; Briggs and Deeming 2016; Akresh *et al.* 2017). For example, the Common Blackbird *Turdus merula*

exhibit variation in nest wall thickness and in the mass of dry grass used within the nest (Mainwaring *et al.* 2014b). Pied Flycatchers *Ficedula hypoleuca* also show variation in the leaf species used within their nests (Briggs and Deeming 2016). Walsh *et al.* (2010) showed that individual Southern Masked weaver *Ploceus velatus* and Village weavers *Ploceus cucullatus* males built nests that decreased in size as more nests were built. Racial differences in nest construction have been reported for White-Crowned Sparrows *Zonotrichia leucophrys* sub-species when nesting at different altitudes (Kern 1984). Despite similarities in body mass between the different subspecies, *Z. l. oriantha* nests were the greatest in mass compared to *Z. l. leucophrys* and *Z. l. nuttalli* nest masses (Kern 1984). White- Crowned Sparrow nests built at higher elevation were larger than those built on the ground (Kern 1984).

Data for the components of nest composition are scarce in the literature, which has hindered the development of understanding the relationship between nest construction and function (Deeming and Mainwaring 2015). For some species, where quantitative data for nest composition are available, it is unclear whether it is representative of the species; for example, Elts (2005) reported on the materials used to construct Willow Warbler *Phylloscopus trochilus* nests by studying only a single nest. By contrast, recent studies that have investigated nest construction typically have a larger sample size (n≥8) which may allow for a better representation of species specific nest construction than a single nest (Britt and Deeming 2011; Mainwaring *et al*. 2014b; Biddle *et al.* 2015; Taberner-Cerezo and Deeming 2016; Gray and Deeming 2017). The growing knowledge base for nest materials suggests that there is great interspecific variation in the specific type and amount of nest materials used within nests. For example, 41% of the nest mass of Long-Tailed Tits *Aegithalos caudatus* was contributed to by feathers (McGowan *et al*. 2004) whereas Common Blackbird nests were constructed entirely with plant derived materials (Biddle *et al.* 2015). Intraspecific variation in nest composition has also been reported in species such as Common Blackbirds which have been shown to vary the mass of grass within the cup lining (Mainwaring *et al*. 2014b). The use of leaves in individual Pied Flycatcher *Ficedula hypoleuca* nests was thought to be opportunistic rather than selective and reflected the geographical location (Briggs and Deeming 2016). Quantifying nest materials may also allow insights into avian behaviour and the influence of human activities on avian behaviour due to the

incorporation of artificial materials into nests. The incorporation of artificial materials within nests has been reported for several species, for example, Black Kites *Milvus migrans* used white plastic within their nests (Sergio *et al*. 2011). The use of artificial "wool" in Great Tit *Parus major* nest lining was also opportunistic as there was no obvious colour preference of the wool-like substance collected by the birds and birds did not collect any material more than 200m away from the nest (Surgey *et al.* 2012). Cigarette butts found in House Sparrow *Passer domesticus* and House Finch *Carpodacus mexicanus* nests from urbanized areas have been shown to play an anti-parasitic role (Suarez-Rodriguez *et al.* 2013). Building on the knowledge base of quantified nest materials should help develop a clearer understanding of the interspecific and intraspecific variation that may exist in the wide diversity of avian nests.

Nests may also be separated into different structural regions; typically an outer region consisting of coarse materials and an inner region of softer, finer material lining the cup (Hansell 2000). Previous studies have suggested that animal derived materials are more common within the cup lining than the outer nest (Deeming and Mainwaring 2015), perhaps due to the high insulative properties of materials, such as feathers (Hilton *et al*. 2004). Animal derived materials, such as hair and feathers may also provide cushioning for eggs and chicks (Healy *et al.* 2015). Although Hilton *et al.* (2004) showed that grass was relatively poor insulating material, grass is the main component lining Blackbird nest cups, which lack animal derived materials (Ferguson-Lees *et al.* 2011; Mainwaring *et al.* 2014b; Biddle *et al.* 2015).

1.2. Nest function

The role of the nest in incubation has been of interest in recent years (Deeming 2002, 2013, 2016). Incubation of avian eggs can be defined as providing the developing embryos with optimal levels of temperature, humidity, gaseous exchange needed for successful development and completion of incubation (i.e hatching of offspring) (Ar and Sidis 2002; Deeming 2016). In some species, turning of the eggs is also required for successful incubation (Deeming 2002, 2016). In non-passerines, the nest acts as the location for incubation where the bird acts as the incubator (Deeming 2016). In smaller passerines, the nest functions in conjunction with the bird to achieve successful incubation of the clutch, i.e. the bird-nest

incubation unit (Deeming 2016). For passerines in general, the relative size of the eggs and the nest to the mass of the bird is greater than in non-passerines (Deeming 2013, 2016). The nest may need to be large enough to compensate for the relatively small size of the bird to allow for successful incubation (Deeming 2016). The passerine nest may therefore be constructed by the bird to allow the nest to aid successful incubation alongside the bird by being built for optimal conditions for successful embryonic development and hatching of the clutch (Deeming 2016).

Optimal development of passerine embryos occurs at temperatures between 36-45°C (DuRant *et al.* 2013) thus it would be expected that nest microclimate is essential to the successful incubation and hatching of avian eggs (Ar and Deeming 2009). Successful incubation relies on effective thermoregulation of the eggs, sufficient movement of respiratory gases from the eggs, a suitable nest humidity and the ability to turn eggs if needed (Ar and Deeming 2009). Contact incubation is relatively well studied in terms of thermal regulation of eggs and the heat transfer from the brood patch to the clutch (Lea and Klandorf 2002; Turner *et al.* 2002; DuRant *et al.* 2013). The importance of maintaining optimal nest microclimate is supported by evidence that suboptimal nest temperatures can negatively affect embryo and nestling development (Webb 1987; Rodriguez *et al.* 2016a, 2016b). Developing chicks need to experience appropriate thermal conditions in order to reduce the harmful impact of either hyperthermia or hypothermia (Rodriguez *et al.* 2016a, 2016b). There is also evidence of the importance of optimal nest thermoregulation for post-fledgling success in passerines (Greño *et al.* 2008; Rodriguez *et al.* 2016a, 2016b). Hypothermia may cause the developing chick to invest more resources into thermoregulation, consequently compromising growth processes and the propagation of the immune system (Rodríguez and Barba 2016a, 2016b). Due to offspring survival directly affecting reproductive success of a parent it would therefore seem beneficial to adapt a nest to mitigate the negative effects of suboptimal environmental temperatures.

Nest insulation has been considered a key factor influencing the nest microclimate which in turn may impact reproductive success and offspring survival (McGowan *et al.* 2004; Deeming and Mainwaring 2015; Deeming and Pike 2015; Deeming and Biddle 2015; Nord and Williams 2015; Deeming and Gray 2016a; Gray and Deeming 2017). Nest morphology seems to influence the thermal

properties of the nest in some species at least by determining the level of insulation the structure can provide (Reid *et al.* 2002a; Hilton *et al.* 2004; Mainwaring *et al*. 2012; Tulp *et al.* 2012; Deeming and Biddle 2015; Akresh *et al.* 2017; Gray and Deeming 2017, Biddle *et al.* 2018).

Multiple methodologies have been used in previous studies to investigate nest thermal properties. The use of multiple methodologies to investigate nest thermal properties may potentially cause difficulties when comparing nest thermal properties between studies although such methodologies have advanced over time. Palmgren and Palmgren (1939) are assumed to be the first to investigate nest thermal properties, which they inferred by measuring the rates of cooling of objects placed inside nests. Later studies investigated nest thermal conductance ($W \cdot m^{-2} \cdot °C^{-1}$), the rate of heat flux, the flow of heat energy per unit time, moving through the nest wall, per temperature unit, although methodologies also vary for this (Heenan and Seymour 2011, 2012). For example, Whittow and Berger (1977) measured heat flux of heated water within a flask place in Hawaii amakihi *Hemignathus virens* nests whereas Skowron and Kern (1984) measured thermal conductance of various North American passerine nests containing water-filled balloons. Heenan and Seymour (2011, 2012) measured thermal conductance of Australian passerine nests using a steady state system by placing an egg heater in nests and measuring the heat flow out through the wall. Other studies have investigated cooling rates of objects within nests and nest insulation to infer nest thermal properties. Ar and Sidis (2002) placed a heated steel ball inside Common Blackbird nests and measured the cooling rates. Hilton *et al*. (2004) measured the cooling rates of domestic fowl *Gallus gallus* eggs inside artificial nests to investigate the effect of feathers on nest insulation.

In recent years, technological advances have enabled the development of further methods to test the thermal properties of nests (Smith *et al.* 2015). Boulton and Cassey (2012) used thermography, a method of detecting infrared radiation using thermal imaging cameras, to measure heat loss from Great Tit eggs during off bouts in incubation. Deeming and Pike (2015) used infrared (IR) thermography to investigate the thermal profile of Blue Tit and Great Tit nests. Digital temperature dataloggers were used by McGowan *et al.* (2004) to measure the nest insulation quality ($°C \cdot 20\ s^{-1}$), otherwise referred to as insulatory values, of Long-Tailed Tit nests. Later studies investigated insulatory values of other passerine nests also using temperature loggers but the placement of the temperature loggers within the

nests varies between studies (Mainwaring *et al.* 2012, 2014b; Deeming and Biddle 2015; Deeming and Gray 2016b; Taberner Cerezo and Deeming 2016; Gray and Deeming 2017; Biddle *et al*. 2018). In several studies the temperature logger was pushed inside the nest material in the middle of the cup (Mainwaring *et al.* 2012, 2014b; Deeming and Biddle 2015; Taberner Cerezo and Deeming 2016). Deeming and Gray (2016b) inverted the nest and elevated the temperature logger to allow the logger to rest on the cup rather than force it into the materials, which has been subsequently used in other studies (Smith *et al.* 2015; Deeming and Gray 2016b; Gray and Deeming 2017; Biddle *et al*. 2018). The use of the two different methodologies means that cross-study comparisons of insulatory values and cooling rates should be taken with care.

Multiple studies investigating the thermal properties of nests have been conducted under laboratory conditions, which whilst allowing for controllable conditions, do not provide a full representation of the thermal properties of the nest in the natural environment (Heenan and Seymour 2011, 2012; Mainwaring *et al*. 2012, 2014b; Deeming and Biddle 2015; Deeming and Gray 2016a; Gray and Deeming 2017; Biddle *et al.* 2018). However, studies have tested the effect of air movement on nest thermal properties in order to develop a better understanding of nest function in the natural environment (Palmgren and Palmgren 1939; Heenan and Seymour 2012; Gray and Deeming 2017). During an *in situ* study, windy conditions increased cooling rates inside Common Rosefinch *Carpodacus erythinus* nests by 144% and 191% for Chaffinch nests *Fringilla coelebs* (Palmgren and Palmgren 1939). Heenan and Seymour (2012) investigated the difference in thermal conductance of nests from two Australian passerines when exposed to air movement. In order to control for different wind speeds, Heenan and Seymour (2012) constructed a wind tunnel, and at maximum airspeed (0.88 ms^{-1}) the thermal conductance of nests increased by 170%. Gray and Deeming (2017) also adopted the method of using a similar wind tunnel design to investigate the effect of air movement on passerine nests but measured nest insulation and cooling rates and used a fixed but faster airspeed of 1.5 m s^{-1}. During periods of air movement, internal cooling rate increased on average by 156% for Motacillidae and Fringillidae nests compared to still air conditions (Gray and Deeming 2017).

Hole-nesting species have been the focal species in many studies that have investigated the thermal properties of passerine nests, possibly due to the ease of

locating and studying birds within nest boxes (Mainwaring *et al.* 2014a; Deeming and Mainwaring 2015). Fewer studies have chosen to study open-cup nesting species despite the likely possibility that cavity nesting and open-cup nesting species experience different selection pressures (Lamprecht and Schmolz 2004; Mainwaring *et al*. 2014b; Akresh *et al.* 2017; Gray and Deeming 2017). Open-cup nests may be more vulnerable to changes in environmental conditions, as parent birds and offspring are more exposed to various weather conditions than hole nesting species, thus open nests may also be designed differently to cavity nests due to variation in selection pressures (Lamprecht and Schmolz 2004; Mainwaring *et al*. 2014b).

Nests may also be built to mitigate adverse effects of precipitation and moisture on the clutch and chicks (Wesolowoski 1996; Wesolowoski *et al*. 2002; Heenan 2013; Öberg *et al.* 2015). Precipitation and rainfall levels have been shown to reduce the survival and reproductive fitness of both parents and offspring in Northern Wheatears *Oenanthe oenanthe*. Fledgling success was negatively correlated with rainfall and the magnitude of the effect on fledgling success varied depending on the time from hatching (Öberg *et al.* 2015). Although direct investigation into the hydrological properties of nests are rare, studies have considered nest construction in terms of preventing nest soaking (Wesolowoski 1996; Wesolowoski *et al*. 2002). Wesolowski (1996) suggested that Marsh Tits *Poecile palustris* constructed their nests with water-absorbing layers of moss to create “bulky foundations” in order to prevent tree sap reaching the centre of the nest. Weaver Birds have been shown to build nests with overlapping leaves which may allow rainwater to flow off the nest roof (Crook 1960, 1963). Similar to the study of nest thermal properties, the hydrological properties of nests have been mainly considered for cavity nesting species (Wesolowoski 1996; Wesolowoski *et al*. 2002). Cavity nesting species may be less vulnerable to direct rainfall and environmental humidity than open-cup nesting species but perhaps likely to be more vulnerable to nest soaking from free-flowing tree sap (Wesolowoski 1996; Wesolowoski *et al*. 2002).

In the laboratory, Slagsvold (1989b) investigated the water retention of open cup nests from six passerine species by submerging nests in water for 1 minute and found that some species dry out faster, or return to original dry mass faster than others. It was proposed that differences in nest materials and nest structure

influenced water retention. For example, the light, less dense nests made of grass such as those built by Eurasian Blackcaps *Sylvia atricapilla* dried faster than dense thrush nests (Slagsvold 1989b). Rohwer and Law (2010) submerged Yellow Warblers *Dendroica petechia* nests from two different geographical locations and measured the water absorption and drying times of nests (Rohwer and Law 2010). Nests built in more northerly regions absorbed more water and dried out slower than southern Yellow Warbler nests despite the former having lower rates of precipitation. Further investigation into the hydrological properties of open cup nests could allow insights into the relationship between construction and function in terms of mitigating the adverse effects of water.

1.3. Focal species

The focal species of this thesis are three closely related members of the Old World warblers (Sylviidae): Willow Warblers *P. trochilus*, Eurasian Reed Warblers *Acrocephalus scirpaceus* and Eurasian Blackcaps *S. atricapilla*. All are migratory species, breeding in Europe during the summer and typically winter in Sub-Saharan and Tropical Africa (Tiainen *et al.* 1983; Cramp 1992; Leniowski and Węgrzyn 2014; Morrison *et al.* 2015).

The three species exploit different nest sites (Tianen *et al.* 1983; Cramp 1992; Leniowski and Węgrzyn 2014; Morrison *et al.* 2015). Willow Warblers are ground nesting species that build nests under vegetative cover whereas both Reed Warbler and Blackcaps build nests above ground (Cramp 1992). Reed Warblers nest among reed beds, typically *Phragmites australis*, where nests are suspended between two or three reed stems (Cramp 1992; Honza *et al*. 1998). Blackcaps nest in shrubs and trees in woodlands (Storch 1998). Blackcaps and Reed Warblers build open cup nests, whereas Willow Warbler nests are built with a roof and an entrance to the side (Tiainen *et al.* 1983; Cramp 1992; Leniowski and Węgrzyn 2014).

The three species differ in the type of parental care delivered to offspring and the sex of the nest builder also differs between the species. Uniparental care is delivered by female Willow Warblers, which have 74.3% incubation attentiveness and the female who is also the sole builder of the nest (Cramp 1992). Reed Warbler males have been reported to aid females with construction and parental care, but females are the main caregiver with an attentiveness during incubation of 68.9% (Cramp 1992). Blackcap males build a series of cock nests, which a female may adopt and develop into the functional nest for incubation (Cramp 1992). Blackcaps

exhibit 100% biparental care; males contribute to 45% of contact incubation and can develop a brood patch (Cramp 1992, Redfern 2010). Deeming and Mainwaring (2015) suggested that if nest building is shared between the sexes then incubation is also shared. Where data were available, 22 warbler species exhibited shared nest building and incubation. Of these species, females exclusively construct the nest and incubate in 17 species (Cramp 1992). Contrary to the suggestion by Deeming and Mainwaring (2015), in 7 warbler species, both sexes share nest construction, but the female incubates and in 4 species the female builds and both sexes share incubation (Cramp 1992). *Sylvia* males reportedly build cock nests and exhibit shared nest construction and incubation (Cramp 1992). Females are the only nest builders and incubators in *Phylloscopus* warblers, where data were reported (Cramp 1992). Proportion of shared parental care is varied for other warbler genera (Cramp 1992).

Studying these three species will allow further insight into the extent of interspecific variation in nest morphology within a family, which has only previously been done for thrushes and finches (Biddle *et al.* 2018). Data for nest materials are limited for Sylviidae species; data for one Willow Warbler nest is available (Elts 2005) and feather mass, but no other materials, has been quantified for twelve Willow Warbler nests (Tiainen *et al.* 1983). Descriptions and feather masses are reported for Finnish Wood Warbler *Phylloscopus sibilatrix* and Chiffchaff *Phylloscopus collybita* nests (Tiainen *et al*. 1983). The three species of Warblers studied in this thesis can provide data to be added to the growing knowledge base of nest composition.

Many studies investigating the function of nests have focussed on cavity nesting species, such as Blue Tits and Great Tits, but fewer studies have investigated the function of open-cup nests (Mainwaring *et al.* 2014a; Deeming and Mainwaring 2015). Open cup nests and cavity nests may experience variation in exposure to weather conditions thus may differ in construction and function. Here, the thermal and hydrological properties of non-cavity nesting warbler species were investigated to add to the developing understanding of nest functionality.

1.4. Aims and objectives

Overall, this thesis provides empirical data for the relationship between nest construction and function of closely related passerines from the Old World warblers

(Sylviidae). Chapter 2 builds on the growing knowledge base of quantified nest composition for multiple avian species and investigates the intra- and interspecific variation in nest morphology between closely related species, in both nest size and the materials used within nests. Whether nest regions are identifiable by the type and quantity of nest materials used is also investigated.

In Chapter 3, the thermal properties of warbler nests are investigated to measure insulatory values of nests under a variety of conditions. The study investigates the effect of nest dimensions and nest composition on the insulative properties of the nests. It is predicted that nests with greater dimensions and a greater mass of grass and animal derived materials will be more insulating. The hypothesis that air movement can affect the insulative properties of nests and can affect the internal microclimate is also tested. As reported by Gray and Deeming (2017), the internal microclimate of Fringillidae and Motacillidae nests was affected by air movement but overall insulatory value of the nests increased. Thus, it is predicted that warbler nests can act as a buffer against greatly increased rates of cooling inside the nest during periods of air movement, but internal cooling rates will increase in comparison to still air conditions. The influence of test location (the location where trials are conducted, either in an open laboratory or enclosed chamber) on data from thermal trials is also investigated to provide suggestions towards developing a standardised method for thermal trials.

The relationship between nest design and function is explored further in Chapter 4, which investigates the hydrological properties of warbler nests. Interspecific variation in nest hydrological properties, and whether nest dimensions and composition contribute to their hydrological properties are investigated.

The final chapter provides an oversight of the nests of the three warbler species and applies the construction of their nests into a broader context of avian reproduction.

Chapter 2: Interspecific variation in nest morphology of Sylviidae nests.

2.1. Introduction

Nest building is a behaviour displayed by a multitude of avian species, although there is interspecific and intraspecific variation in nest design (Hansell 2000, Mainwaring *et al.* 2014a). Understanding of the design and function of avian nests has increased greatly within recent years (Mainwaring *et al.* 2014a; Deeming and Mainwaring 2015). The primary function of a nest is to provide a receptacle for eggs during incubation, and in many cases, chick rearing. Recent developments in research have pointed to a multifunctional role of nests, including roles in sexual signalling, thermoregulation of nest microclimate, protection from predators, pathogenic and parasitic defence (Deeming *et al.* 2012; Moreno 2012; Mainwaring *et al.* 2012, 2014b, 2014a; Biddle *et al*. 2015, 2016, 2017; Heenan *et al.* 2015; Deeming and Gray 2016; Soler *et al.* 2017).

The dimensions of the nest and the specific materials used within the nest may help determine the function of the nest (Mainwaring *et al.* 2012; Deeming 2013; Biddle *et al.* 2015; Taberner Cerezo and Deeming 2016; Soler *et al.* 2017). The amount of insulating material present in the nest has been linked to the successful incubation and survival of chicks (Mainwaring *et al*. 2012, 2014b; Biddle *et al.* 2015; Heenan *et al*. 2015; Taberner Cerezo and Deeming 2016). Presence of feathers within a nest is associated with high levels of insulation, slower cooling of eggs and increased nestling growth rates in passerines (Lombardo *et al.* 1995; Windsor *et al.* 2013; Hilton *et al.* 2014). A direct link has been suggested between nest material composition and telomere length in Spotless Starlings *Sturnus unicolor* indicating that the nest materials selected by the parents have an influence on offspring development (Soler *et al.* 2017).

Empirical data for the quantity and variation of materials used to construct a nest is limited to a few (i.e. < 20) species (Rohwer and Law 2010; Britt and Deeming 2011; Álvarez *et al.* 2013; Mainwaring *et al.* 2014b; Biddle *et al.* 2015; Deeming and Mainwaring 2015; Taberner Cerezo and Deeming 2016; Briggs and Deeming 2016; Biddle *et al.* 2017; Biddle *et al.* 2018). Many descriptive studies only focus on a few examples of nests from a single species, thus a typical representation of nest composition for a species is difficult to obtain. Developing an understanding of the relationship between nest construction behaviour and function requires additional

quantitative data for nest composition. Alongside this, the basis for the selection and the function of nest materials is not yet fully understood, therefore further quantification of materials used by multiple species is needed.

The notion that nest building is a plastic and adaptable behaviour, rather than an entirely instinctive or fixed behaviour has become more accepted in recent years (Britt and Deeming 2011, Deeming *et al.* 2012; Mainwaring *et al.* 2012, 2014a; Bailey *et al.* 2014a, 2014b, Healy *et al.* 2015; Briggs and Deeming 2016). Current evidence suggests that there is a high degree of interspecific variation in nest design, i.e. nest dimensions and nest materials used may differ greatly between species (Britt and Deeming 2011; Biddle *et al*. 2015, 2017, 2018). Studies have also reported intraspecific differences; nests built by individuals of the same species differ in both dimensions and quantity of nest materials, and some of this variation is related to differences in geographical locations of nests (Rohwer and Law 2010, Deeming *et al.* 2012; Mainwaring *et al.* 2012, 2014b, Briggs and Deeming 2016). Mainwaring *et al.* (2012) found that Blue Tit *Cyanistes caeruleus* and Great Tit *Parus major* nests built in locations with increased environmental temperatures had a lower cup lining mass. Intraspecific variation has been seen in the American Yellow Warbler *Dendroica petechia*, in which nests built at different latitudes had morphological differences (Rohwer and Law 2010). McGowan *et al.* (2004) showed that nests built by Long-Tailed Tits varied in the mass of feathers used throughout the breeding season. Common Blackbirds can also alter the mass of dry grass used within the cup (Mainwaring *et al.* 2014a; Biddle *et al.* 2015). Many studies that have investigated intraspecific variation in nest building behaviour have found that birds adapted their behaviour to build nests with suitable microclimate when weather conditions changed with latitude and seasonal changes (McGowan et al. 2004; Rohwer and Law 2010; Mainwaring *et al.* 2014a; Biddle *et al.* 2015).

Nest composition may vary within the nest because nests may have defined regions, such as an outer nest and cup lining (Hansell 2000; Biddle *et al.* 2015, 2017). Common Blackbird nests are built with an outer structure, a mud cup and a cup lining layer (Biddle *et al*. 2015). The physical properties of nest materials used within different nest regions also varied, suggesting birds can select materials for a specific function within the nest (Biddle *et al*. 2017). Outer nest structures may play a role in crypsis, reduction in adverse effects from environmental conditions and provide structural support (Hansell 2000; Biddle *et al.* 2015). Cup lining materials

may primarily function in thermoregulation of the clutch and provide cushioning for the eggs (Hansell 2000). Cup lining materials, such as feathers may also be vital for repulsion of parasites and pathogenic defence (Winkler 1993; Mainwaring and Hartley 2008).

In this study, the nest dimensions and the quantity and quality of materials used in nest construction were determined from three warbler species to test the hypothesis that nests built by closely related warbler species are significantly distinct in the quantity and type of nest materials used within the nest. In addition, it was hypothesised that the nest materials used to construct the outer nest and cup lining would be different and so were compared within each species.

2.2. Materials and Methods

Fifty-six Sylviidae nests: 23 Willow Warbler *Phylloscopus trochilus*, 21 Reed Warbler *Acrocephalus scirpaceus* and 12 Blackcap *Sylvia atricapilla* were studied.

Forty-three nests were collected by volunteers from the British Trust for Ornithology from various locations within the UK. Thirteen Reed Warbler nests were collected from Wicken Fen, Cambridgeshire by D. C. Deeming and N. Davies. The nests collected by all parties had been monitored during breeding and collected at the end of the breeding season (May-August) during 2014, 2015 and 2016 (see more details in Appendix. 1). Upon arrival at the University of Lincoln, all nests were frozen for 72 hours at -20ºC to kill parasites and pathogens present in the nests (Britt and Deeming 2011). All nests were stored dry in plastic bags within cardboard boxes and kept at room temperature. Nests did not visibly deteriorate or alter in morphology post-storage.

The physical dimensions of each nest were measured and recorded in June 2016. Nest mass was determined using a digital balance (A & D Company Limited, model FX-3000i). Nest dimensions were measured with Mitutoyo digital callipers, accurate to ± 0.02 mm (Biddle *et al.* 2016, Gray and Deeming 2017). The following measurements were taken: Total nest diameter - long and short axis (mm), cup diameter - long axis and short axis (mm), average width of each wall, repeated three times, was used for nest wall thickness (mm), cup depth (mm), cup volume (cm^3) and nest height (mm). Base thickness (mm) was derived from the difference between cup depth and nest height. Cup volume (cm^3) was determined by lining the

cup with cling-film and filling the cup with 5mm glass beads, the total mass of beads was then multiplied by a pre-determined density (Biddle *et al.* 2015, 2017).

Prior to deconstruction, nest regions were identified for each nest (Biddle *et al*. 2017). The outer-nest was identified as the external walls of the nests with mainly plant-material, such as stems, moss and coarse grasses. The cup was identified as the innermost, "cup-shaped" region within the nest. Nest deconstruction quantified materials for a subset of nests: 12 Blackcap, 10 Reed Warbler and 8 Willow Warbler nests. The remaining 26 nests were not deconstructed due to time constraints. Individual nest components were identified and extracted using laboratory tweezers. The total mass of the nest was determined by summing all of the materials deemed to be used in the nest and excluding other materials, e.g. insects, faeces, pulli bones, that would have been added during incubation and rearing (Britt and Deeming 2011, Biddle *et al.* 2017). The total weight of individual components for cup materials and outer nest materials were quantified using digital scales.

Most statistical analyses were conducted using Minitab version 17. A Levene's test was used to confirm a departure from homogeneity within the composition data. Analysis of variance (ANOVA) was used to determine interspecific differences in nest dimensions. Post-hoc Tukey's HSD was applied to significant outputs ($P<0.05$). Principal component analysis was conducted to assess interspecific variation between the mass of materials used within the whole nest. Materials selected for principal component analysis were those present within the nest when deconstructed and categorised into the following; grass, moss, hair, feather, stems, roots, leaves, lichen, artificial materials and invertebrate silk. A Kruskal-Wallis test was conducted to determine the effect of species on the average values of the principal components.

Discriminant analysis to determine which materials could be used to identify the cup from the outer nest was performed using SPSS 21.0 statistical package (IBM Corp., New York). Data for mass of nest components within the nest cup and outer nest were not normally distributed thus were converted to normal values using arcsin transformation before analysis (Britt and Deeming 2011).

2.3. Results

Nest masses for all species (example nests are shown in Fig. 2.1.) were significantly different with Willow Warbler producing the heaviest of the three species, whereas Blackcap nests had the smallest mass (Table 2.1). Cup volume was greatest for Willow Warblers but smallest for Reed Warblers. Nest diameter, cup diameter and wall thickness were greatest for Willow Warbler nests whilst Reed Warbler nests were the smallest (Table 2.1). By contrast, nest height, cup depth and floor thickness were greatest for Reed Warbler nests (Table 2.1). Cup depth was not significantly affected by species but base thickness was significantly different between Reed Warbler and Blackcap nests, and Willow Warbler and Blackcap nests. Nest height was significantly different between all species (Table 2.1).

Table 2.1. Descriptive statistics of female body mass (mean and range) as reported by Cramp (1992) and nest dimensions (mean ± SD) for Willow Warbler, Reed Warbler and Blackcap nests. ANOVA results for the effect of species on nest dimensions are provided (*F*- value with *P*-value). Superscripts indicate significant differences within rows at P <0.05 as indicated by Post-hoc Tukey's HSD.

Nest dimension	Willow Warbler (N = 23)	Blackcap (N = 12)	Reed Warbler (N = 21)	$F_{2,53}$ (p-value)
Female body mass (g)	8.8 (6.1-10.0)	18.9 (16.9-22.2)	11.1 (9.6-12.4)	
Nest mass (g)	18.9 ± 3.7^{A}	7.6 ± 2.6^{B}	11.3 ± 3.1^{C}	56.28 (<0.001)
Cup volume (cm^3)	91.4 ± 48.5^{A}	$68.5 \pm 5.4^{A,B}$	63.8 ± 14.9^{B}	4.34 (0.018)
Cup depth (mm)	40.6 ± 14.8	33.3 ± 7.9	42.2 ± 8.1	2.48 (0.093)
Nest height (mm)	64.9 ± 20.1^{A}	45.4 ± 9.4^{B}	67.9 ± 14.6^{A}	7.96 (0.001)
Nest diameter (mm)	125.9 ± 16.8^{A}	106.4 ± 13.3^{B}	78.3 ± 5.9^{C}	74.69 (<0.001)
Cup diameter (mm)	79.7 ± 16.1^{A}	66.2 ± 7.2^{B}	55.2 ± 5.3^{C}	25.64 (<0.001)
Wall thickness (mm)	31.7 ± 5.9^{A}	18.1 ± 3.9^{B}	11.6 ± 1.4^{C}	49.11 (<0.001)
Base thickness (mm)	24.4 ± 11.4^{A}	12.1 ± 5.8^{B}	25.7 ± 13.0^{A}	6.43 (0.003)

Table 2.2. Mean (± standard deviation) mass of nest materials present in total nest, cup lining and outer nest for a subset of warbler nests.

Material (g)	Nest region	Willow Warbler (N = 8)	Blackcap (N = 12)	Reed Warbler (N = 10)
Grass	Total	5.1 ± 2.04	3.1 ± 1.5	7.9 ± 2.3
	Cup lining	3.5 ± 1.2	1.2 ± 0.60	3.9 ± 0.66
	Outer nest	1.6 ± 1.1	1.9 ± 1.3	4.0 ± 2.2
Fern	Total	0.30 ± 0.44	-	-
	Cup lining	0.06 ± 0.09	-	-
	Outer nest	0.24 ± 0.35	-	-
Moss	Total	4.2 ± 1.5	0.12 ± 0.19	0.17 ± 0.29
	Cup lining	0.60 ± 0.33	0.03 ± 0.06	0.01 ± 0.03
	Outer nest	3.6 ± 1.4	0.09 ± 0.14	0.16 ± 0.27
Hair	Total	0.71 ± 0.61	0.24 ± 0.34	0.16 ± 0.20
	Cup lining	0.67 ± 0.58	0.17 ± 0.26	0.03 ± 0.05
	Outer nest	0.04 ± 0.03	0.07 ± 0.11	0.13 ± 0.16
Feather	Total	0.78 ± 0.80	0.003 ± 0.009	0.01 ± 0.03
	Cup lining	0.72 ± 0.79	-	-
	Outer nest	0.06 ± 0.05	0.003 ± 0.009	0.01 ± 0.03
Stems	Total	0.91 ± 1.4	0.78 ± 1.29	-
	Cup lining	0.07 ± 0.09	0.07 ± 0.19	-
	Outer nest	0.84 ± 1.4	0.71 ± 1.1	-
Thorn	Total	0.09 ± 0.14	0.16 ± 0.41	-
	Cup lining	0.04 ± 0.08	-	-
	Outer nest	0.05 ± 0.06	0.16 ± 0.41	-
Leaves	Total	0.05 ± 0.10	0.09 ± 0.16	0.011 ± 0.03
	Cup lining	0.01 ± 0.02	0.01 ± 0.02	0.004 ± 0.01
	Outer nest	0.04 ± 0.10	0.08 ± 0.16	0.007 ± 0.02
Roots	Total	0.44 ± 0.70	0.35 ± 0.50	0.01 ± 0.15
	Cup lining	0.14 ± 0.21	0.32 ± 0.48	-
	Outer nest	0.30 ± 0.50	0.03 ± 0.04	0.01 ± 0.15
Invertebrate silk	Total	-	0.05 ± 0.06	0.02 ± 0.05
	Cup lining	-	-	-
	Outer nest	-	0.05 ± 0.06	0.02 ± 0.05
Lichen	Total	-	0.014 ± 0.05	-
	Cup lining	-	-	-
	Outer nest	-	0.014 ± 0.05	-
Artificial	Total	-	0.23 ± 0.22	0.14 ± 0.22
	Cup lining	-	0.06 ± 0.06	0.01 ± 0.03
	Outer nest	-	0.17 ± 0.17	0.13 ± 0.20

Figure 2.1. Images of typical nests for A) Willow Warbler, B) Blackcap, and C) Reed Warbler as viewed from above.

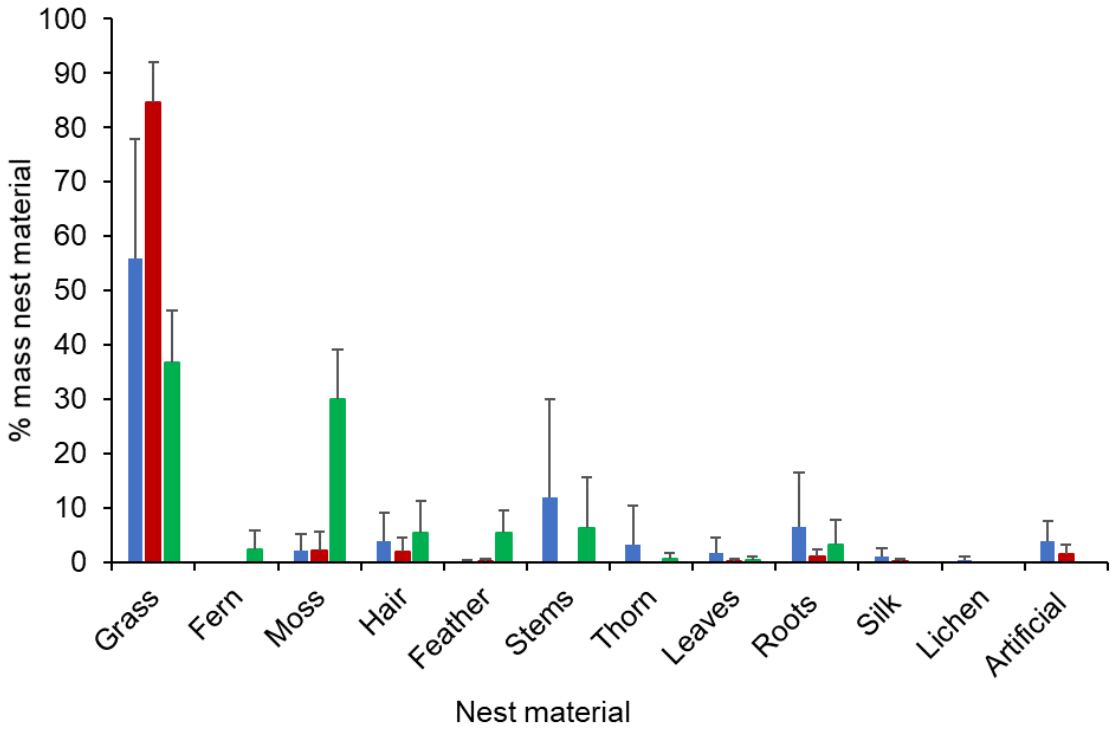

Figure 2.2. Mean (+ SD) Proportion of nest materials present in the whole nest for warbler nests as indicated in Table 2.2. Blue = Blackcap, red = Reed Warbler and green = Willow Warbler.

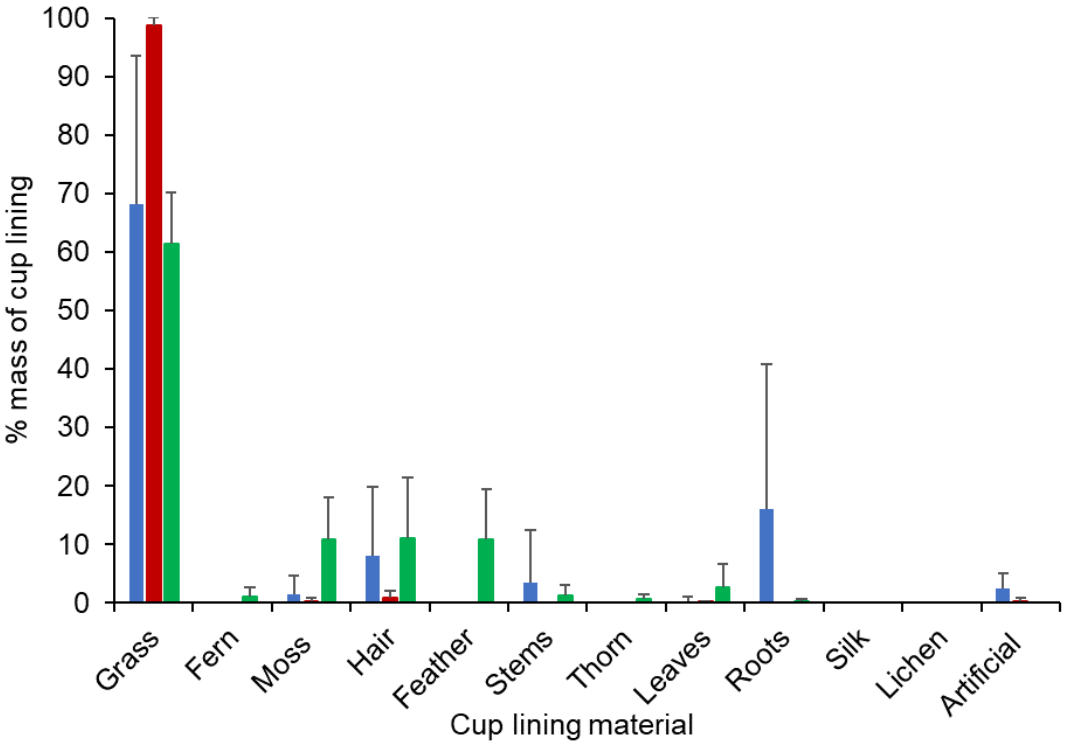

Figure 2.3. Percentage mass (±SD) of nest materials present within the cup lining of 30 warbler nests. Blue= Blackcap, red= Reed Warbler and green= Willow Warbler.

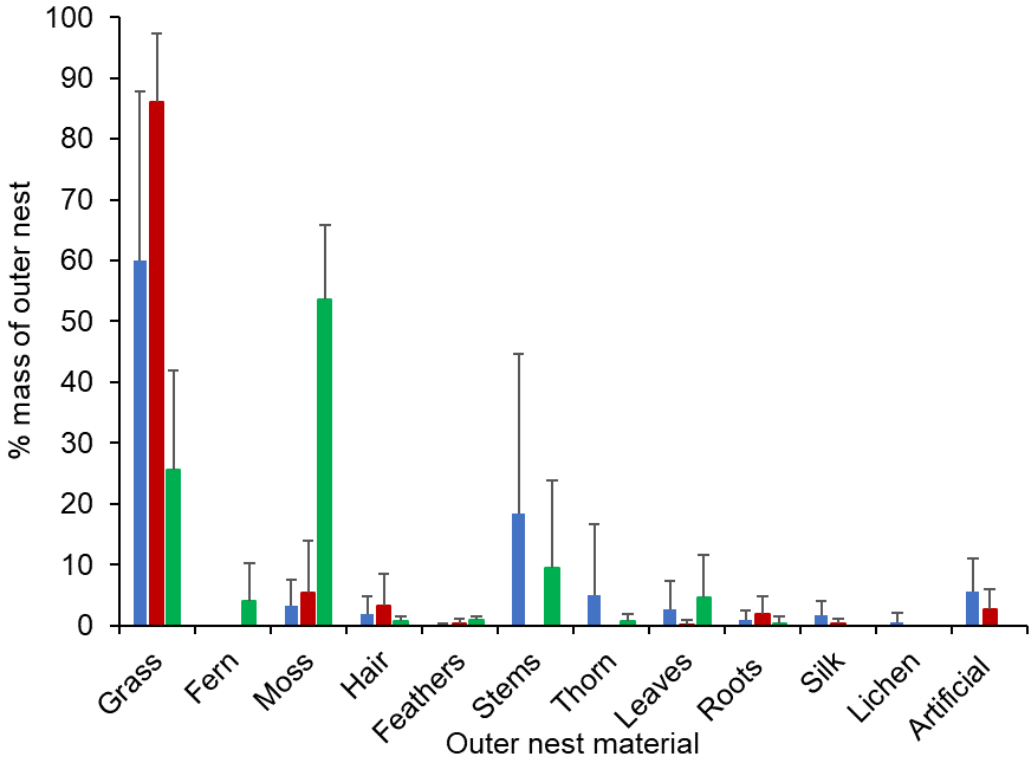

Figure 2.4. Percentage of materials present within the outer nest layer for 30 warbler nests. Blue= Blackcap, red= Reed Warbler and green= Willow Warbler.

Warbler nests were constructed from a variety of plant and animal derived materials and mean values are shown for each type of material in Table 2.2 for total, cup and outer nest components. Given the differences in mean nest mass (Table 2.2) data for each material type were converted into percentage values of the whole nest mass (Taberner Cerezo and Deeming 2015; Briggs and Deeming 2016).

Dry grass was the most common material used within Warbler nests and contributed to 85% of the total mass of Reed Warbler nests (Fig. 2.2) which was the largest percentage mass of any other material contributing to total nest mass. Grass was also the most abundant material in Blackcap and Willow Warbler nests, contributing to 55% and 35% of the nest mass respectively. Dry grass was the main component of Reed Warbler nest cup linings which contributed to 98% of the cup lining mass. Similarly, the cup lining of Blackcap nests was mostly grass however Blackcaps incorporated more stems, roots, hair and artificial materials than Reed Warblers (Fig 2.3). In Reed Warbler and Blackcap nests, grass was present in a greater proportion within the cup than the outer nest. For Willow Warbler nests, 60% of the cup lining mass was grass. The cup lining of Willow Warbler nests also consisted of hair, feather and moss. A greater percentage of the cup was hair in Willow Warbler nests compared to Blackcap and Reed Warbler nests (Fig 2.3). Despite this, blackcap nests had the highest relative proportion of hair in the outer nest (Fig 2.4). Moss was the main component of Willow Warbler structural outer nest walls whereas grass was a major component of the structural outer wall in Blackcap and Reed Warbler nests (Fig 2.4).

Principal component analysis (PCA) revealed that increasing positive values for principal component 1 (PC1) indicated greater mass (g) of leaves, moss and fern present in the nest. Increasing positive values of principal component 2 (PC2) were associated with greater mass (g) of animal derived material within the nest. Willow warbler, reed warbler and blackcap nests are significantly different in terms of nest composition. Willow Warbler nests showed the greatest variability in nest composition whereas Reed Warbler nests showed the least variability in composition. Levene's test confirmed a significant departure of homogeneity for both PC1 ($F_{2,27}$ = 3.47, P = 0.046) and PC2 ($F_{2,27}$ = 10.6, P < 0.001) when species was a factor. Kruskal-Wallis analysis revealed that average values for PC1 and PC2 were significantly different when species was a factor (H = 17.05, DF = 2, *P* < 0.001; H = 7.82, DF = 2, *P* = 0.020, respectively).

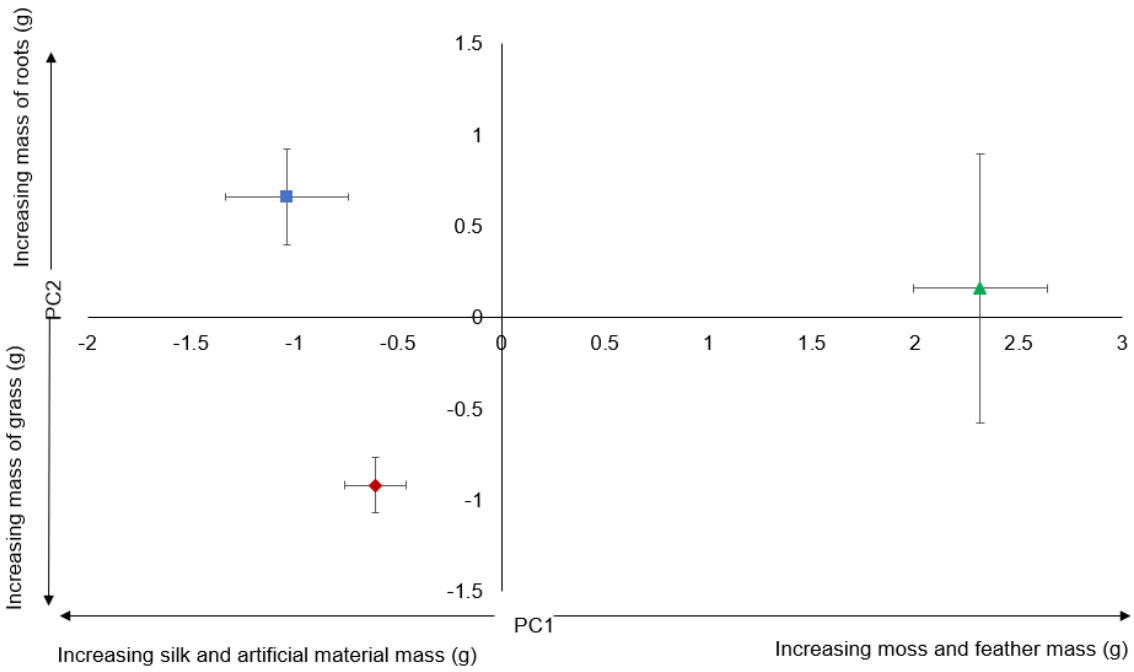

Figure 2.5. Warbler species mean ± SE for PC1 and PC2 values. Blue= Blackcap, red= Reed Warbler and green= Willow Warbler.

Table 2.3. Overall Wilk's lambda and significance values from discriminant analysis for nest materials within warbler nest regions. C represents the cup lining and N represents the outer nest.

	Nest materials				
Species	Grass	Moss	Stems	Invertebrate silk	Wilk's lambda
Willow Warbler		0.169 C<N			0.169 <0.001
Blackcap	0.618 C>N		0.735 C<N	0.532 C<N	0.457 <0.001
Reed Warbler				0.804 C<N	0.804 0.051

Discriminant analysis of data for outer nest materials and cup lining showed that in Willow Warblers, the outer nest and cup could be distinguished by a greater proportion of moss in the outer nest (Table 2.3). Blackcap nest cups are identifiable by a greater proportion of grass in the cup than the outer nest and the outer nest is characterised by a larger proportion of wood and invertebrate silk than the cup. Reed Warbler nests had a greater proportion of invertebrate silk on the outer nest than the cup, but this only approached significance (Table 2.3).

2.4. Discussion

Warbler nests exhibited intra- and interspecific variation in their nest dimensions. Blackcaps built the lightest, shallowest nests whereas Willow Warbler nests were twice the mass of Blackcap and Reed Warbler nests. Interspecific variation was also seen in the composition of Warbler nests in terms of the specific materials used, amount of materials used, and the location of materials within the nest. Willow Warbler nests showed greater intraspecific variation than both Blackcap and Reed Warbler nests.

Previous studies have suggested that intraspecific variation in nest mass can occur with differences in latitude (Rohwer and Law 2010; Crossman *et al.* 2011; Mainwaring *et al.* 2012, 2014b) and habitat differences (Álvarez *et al.* 2013).

Typically, nests built in more northerly regions are heavier than southern nests of the same species (Deeming *et al.* 2012). Great Tit and Blue Tit nests from five different locations in the UK across 580km north to south were heavier in more notherly areas but this was also reflective of lower local temperature in more northely regions of the UK (Deeming *et al.* 2012). Nest mass reported for Willow Warblers in Estonia was greater than the average reported here (Elts 2005). By contrast, Willow Warbler nests from Finland (61°03'N25°03'E) were 4g lighter than that for Willow Warbler nests from the UK even though the UK is to the south of Finland (Tiainen *et al.* 1983). It is not clear what caused the intraspecific variation in nest mass between nests from Finland and the UK, however previous studies have also suggested that year can also influence nest mass (Britt and Deeming 2011).

Nest dimensions reported for warbler nests here are comparable to those for other *Phylloscopus* warblers. In Finland, Wood Warbler *Phylloscopus sibilatrix* and Willow Warbler nests were lighter than Willow Warbler nests reported here but were lighter than nests of the Chiffchaff *Phylloscopus collybita* (Tiainen *et al*. 1983) but it is not clear whether the variation was due to latiduinal or local temperature differences. Reed Warbler nests were the tallest of the three species in this study but were shorter than the larger Great Reed Warbler *Acrocephalus arundinaceus* which may lend itself to interspecific variation in closely related species (Jelínek *et al.* 2016).

The results here are similar to previously reported nest height and diameter for Blackcaps (Storch 1998) at 5cm and 10cm respectively (Table 2.1). Storch (1998) described Blackcap nest walls as “thin” but did not report values. Previous studies have shown that wall thickness correlates with latitude (Rohwer and Law 2010, Crossman *et al.* 2011) and can correlate with the thermal properties of nests from a number of passerine species (Kern 1984; Heenan and Seymour 2012, Akresh *et al.* 2017). Interspecific variation exhibited in nest wall thickness may reflect the variation in insulation and thermoregulatory roles of nests from different species that is needed to achieve successful incubation of the clutch (Deeming 2016). Thicker nest walls may be constructed by birds of a smaller mass in order to minimise heat loss to the external environment during incubation. However, multiple selection pressures such as risk of predation, parasitism, variation in environmental conditions may also be imposed onto species that may influence nest dimensions

(Akresh *et al.* 2017). These aspects of warbler nests will be investigated further in Chapter 3.

Blackcap nests have previously been described as a network of small twigs with visible holes (Storch 1998; Leniowski and Węgrzyn 2014). Leniowski and Węgrzyn (2014) also noted that it was possible to see through the hole in the the base of the nest. Blackcap nests in this study were also loosely constructed and gaps were visible within the nest base and walls. The presence of gaps in the nest structure initially appears counterproductive in terms of nest insulation, but air gaps between nest materials may also provide insulation by trapping a layer of air (Deeming and Biddle 2015). The presence of gaps may also have benefits in ectoparasite control as the gaps may be difficult for the ectoparasites to remain within the nest (Leniowski and Węgrzyn 2014).

Interspecific variation exists in terms of the type and proportion of material used to construct warbler nests. Willow Warbler nests were identifiable by a greater mass of moss and feathers than Blackcap and Reed Warbler nests, with minimal amounts of invertebrate silk and artificial materials. Reed Warbler nests are characterised by a substantial proportion of dry grass with a smaller mass of moss and feathers than Willow Warbler nests.

Willow Warbler nests show the greatest level of variation in composition whereas Reed Warbler nests show the least variation (Fig 2.5). Plasticity in nest construction within species has been demonstrated in other passerine species including Blue Tits and Great Tits (Rohwer and Law 2010; Britt and Deeming 2011; Crossman *et al.* 2011; Mainwaring *et al.* 2014a; Biddle *et al.* 2015). Moreover, plasticity in nest construction may occur due to variation in environmental conditions and the parent birds' ability to assess the properties of the nest materials (Biddle *et al.* 2017). The variation in the mass of grass, moss and feathers between individual Willow Warbler nests may be due to the parent bird's response to the environmental conditions at the nest site. Increased amounts of grass and feathers have previously been associated with better insulated nests therefore in cooler environments it may be expected that the adult female uses more feathers and dry grass (Hilton *et al*. 2004; Mainwaring *et al*. 2012; Mainwaring *et al*. 2014b). The inclusion of feathers in artificial nests has been shown to increase predation rates (Møller 1984). Willow Warblers incorporate feathers into their nest although only 3.8% of the total nest mass is feathers. Feathers may also play a hydrological role within the nest due to

their ability to repel water, which may prevent adverse effects of precipitation on the clutch. The hydrological properties of warbler nests are investigated in Chapter 4.

Previous studies have reported intraspecific variation in nest materials from nests in geographically separate locations (Kern 1984; Mainwaring *et al*. 2012, Mainwaring *et al*. 2014b). The nest materials reported here for Willow Warblers are comparable to reported materials for a single Estonian Willow Warbler nest although 74% of the Estonian nest was made of grass (Elts 2005), whereas average percentage of grass in British nests was 30%. Moss made up 11% of the Estonian nest which is comparable to 23% of the nest mass here. Feathers were present in larger proportion in the Estonian nest than average British nests. The Estonian willow warbler nest contained materials, e.g. bark, wool and artificial materials, that were not present in any Willow Warbler nests reported here. Whether the intraspecific variation shown here are due to factors such as local availability of materials in the geographical location, environmental temperatures or population differences is not clear. However, the difference in the type, mass and proportions of materials used by Willow Warblers in separate locations does emphasise the importance that quantification of nest materials may have in understanding variation in nest building behaviour. Consequently, more research is needed to better understand geographical or climatic effects on nest construction plasticity.

Interspecific variation in nest composition within species of the same genus has previously been reported for *Phylloscopus* warblers (Tianien *et al.* 1983). Feather mass varied between Scandinavian Chiffchaff and Willow Warbler nests (Tianien *et al*. 1983) although whether this is the case for British *Phylloscopus* Warblers has not been explored. Quantification of nest materials for other species of *Sylvia* and *Acrocephalus* Warblers would allow for within genus comparison of nest composition and may provide insights into the evolution of nest construction.

Different regions within avian nests have previously been identified by the types of material present within specific parts of the nest and have been suggested to perform distinct functions (Hansell 2000, Biddle *et al.* 2015, Taberner Cerezo and Deeming 2016). Choice of nest components and specific placement of components in nest regions suggests an awareness of the materials' properties and the environmental conditions by the bird, which has been supported by several studies (Bailey *et al*. 2014a, 2014b; Biddle *et al*. 2015, 2017). The results of this study provide empirical evidence towards the notion that nest materials can be used to

create recognisable nest regions. Willow Warbler and Blackcap nest regions are identifiable by the type and amount of nest materials used to construct the regions. Moss is more common in the outer base and sides of Willow Warbler nests and this is commonly found in the outer nest for many passerine species, and may have functions including tree sap and moisture absorption (Tiainen *et al.* 1983, Wezolowski 1996, Britt and Deeming 2011, Taberner Cerezo and Deeming 2016, Briggs and Deeming 2016).

Blackcap nest cups are distinguishable from the outer nest by a large mass of grass in the cup lining whereas the outer nest has a greater mass of invertebrate silk and stems (Table 2.3). Similarly, dry grass was the most common component lining the cups of European Robin *Erithacus rubecula* nests and was used in a greater proportion within the cup than the outer nest (Taberner Cerezo and Deeming 2016). Stems have also been found in greater proportions in the outer nest built by other passerines. For example, European Robin and Bullfinch *Pyrrhula pyrrhula* nests (Taberner Cerezo and Deeming 2016; Biddle *et al.* 2017). Outer nest material has been associated with coarser material than within the cup lining (Hansell 2000) which is supported here by a greater mass of stems within the outer nest and softer, dry grasses lining the cup which may provide cushioning for the clutch. Dry grass within the nest cup has previously been suggested to provide insulation to the clutch (Mainwaring *et al*. 2014a) which may also be exploited by Blackcaps.

The lack of variation in Reed Warbler nest regions is perhaps reflective of the local availability of nest materials and physical conditions at the nest site. Reed Warblers typically nest within reed beds therefore grasses may be the most available to use and may allow for camouflage. Reed Warblers may avoid the use of animal derived materials to allow for concealment from predators (Møller 1984). Conversely, Reed Warbler nest regions are not distinguishable by the type of material used although invertebrate silk was nearly significantly higher in the outer nest than in the inner nest. Dry grass is used in high proportions in both the cup lining and the outer nest, resulting in similar composition of the outer and cup lining region. Other passerine species such as the Red-Whiskered Bulbul *Pycnonotus jocosus* have been reported to use invertebrate silk on the outer nest walls (Li *et al.* 2015). As invertebrate silk is found in higher proportions on the outer nest it may be used for attachment of the nest to reed stems, tree branches or twigs. The dry grass

used within the cup may potentially be softer and easier to compact than that used in the outer cup providing cushioning for the clutch.

This chapter provides further species data for the growing knowledge base of nest materials in passerines. Additional species data are still needed to further the understanding of nest construction in relation to function. Obtaining and quantifying nest materials from nests of the same species collected from a broad breeding range would also be beneficial to assess the plasticity of nest building behaviours and response of birds to nest building in various geographical locations. Interspecific variation exists in both nest size and nest composition, even within the same passerine family. The reason for this variation is not fully understood. The function of specific nest materials and nest dimensions in benefiting offspring survival is still unclear. Nest material and nest dimension data collated in this chapter will contribute to further chapters exploring the thermal properties and hydrological properties of Sylviidae nests.

Chapter 3: The effect of air movement on the thermal properties of Sylviidae nests

3.1. Introduction

The thermal properties of passerine nests is an area of research that has received growing interest in the last few decades. The increased attention owes itself to interest in developing an understanding the role of the nest in terms of allowing for successful incubation and minimising the energetic costs incurred by the incubating parent birds (Nord and Williams 2015). Therefore, building a nest that can perhaps minimise heat loss and reduce the energetic costs of incubation could be beneficial in increasing offspring fitness and the reproductive success of parents. Despite the growing number of studies (see review by Deeming and Mainwaring 2015), data for the thermal properties of nests remains limited to a few species (McGowan *et al.* 2004; Blackman *et al.* 2006; Deeming *et al.* 2012; Mainwaring *et al*. 2012; Deeming and Gray 2016; Akresh *et al.* 2017; Gray and Deeming 2017; Biddle *et al.* 2018). Deeming and Mainwaring (2015) reviewed nest insulatory values for seven passerine species, which did not reveal any underlying patterns that explain between species variation.

The role of the nest during incubation is not fully understood. Deeming (2002) proposed the "bird-nest incubation unit" i.e. the nest and the bird function together to ensure successful incubation of the clutch. All nests are the location where incubation takes place, but the thermoregulatory role is likely to differ between passerines and non-passerines because of the relationship between female body mass and nest size (Deeming 2016). In larger, non-passerine nests the bird is seen as the incubator of the eggs with the nest serving as a location for incubation but contributing only a small role in temperature regulation (Deeming 2016). In smaller passerines, the nest acts as part of an incubation unit because it is crucial in maintaining a suitable microclimate for incubation of the clutch (Deeming 2016). The microclimate of passerine nests may also be important for chick rearing. In passerines, nest structure and composition varies between species in passerines (Deeming and Mainwaring 2015; Biddle *et al.* 2018) and may therefore be vital in determining nest thermal properties.

Nest design and location may reflect an attempt to mitigate energetic pressures imposed by environmental conditions (McGowan *et al*. 2004; Rohwer and

Law 2010; Mainwaring *et al.* 2012; Deeming *et al*. 2012; Heenan *et al.* 2015; Deeming 2016). In order to build a nest that is thermally beneficial, birds may select an appropriate nest site and materials (Hansell 2000). For example, at high latitudes in the UK, Blue Tit *Cyanistes caeruleus* and Great Tit *Parus major* nests were significantly better insulated than at lower latitudes (Mainwaring *et al.* 2012). Local ambient temperatures may also influence nest structure and composition in Great Tit, Blue Tit and Blackbird nests (Deeming *et al.* 2012; Biddle *et al*. 2015).

Studies *in situ* have investigated the relationship between heat loss and nest design. Pectoral Sandpiper *Calidris melanotos* nests with increased cup and scrape depth exhibited significantly lower heat loss than shallower nests (Reid *et al.* 2002a). The orientation of Orange-tufted Sunbird *Nectarina osea* nests mitigates the adverse effect of solar radiation and wind exposure (Sidis *et al.* 1994). Nests with greater mass are also attributed to colder environmental temperatures during a breeding season (Britt and Deeming 2011; Deeming *et al.* 2012; Mainwaring *et al.* 2012; 2014b). Geographical locations have been found to influence the nest morphology and thermal properties in North American passerines, with nests built in locations that experience higher wind speeds and colder temperatures reducing heat loss significantly better than nests in warmer, stiller conditions (Rohwer and Law 2010; Crossman *et al*. 2011).

The studies described above investigating the thermal properties of bird nests have been conducted in a laboratory under still air conditions. Whilst this is a feasible approach when investigating the basic functional and thermal properties of nests in terms of composition, Deeming (2016) suggested that experiments into nest function should consider all environmental factors that may affect a nest's construction and functional properties. Abiotic factors such as air movement may influence the thermal properties of nests, but few studies have focussed on the effects of air movement on nest construction and function (Sidis *et al.* 1994; Ar and Sidis 2002; Heenan and Seymour 2012; Gray and Deeming 2017). Studies that have exposed nests to moving air have found varying rates of increased heat loss from the nests, ranging from 8% to 190% (Sidis *et al*. 1994; Heenan and Seymour 2012; Gray and Deeming 2017).

This chapter investigates the thermal properties of nests from three species of Old World warblers of the Sylviidae: Willow Warbler *Phylloscopus trochilus*, Reed Warbler *Acrocephalus scirpaceus*, and Blackcap *Sylvia atricapilla*. It was

hypothesised that nest thermal properties are influenced by nest composition and dimensions. To test this idea, thermal measures were studied in still-air conditions within the laboratory and within an enclosed wind tunnel under still air and moving air conditions. It was predicted that: 1) insulatory properties would be positively correlated with nest dimensions and nest materials: 2) moving air would increase nest insulatory value but would still have an adverse effect on the internal nest environment; and 3) species differences would be found in nest thermal properties due to variation in nest design.

3.2. Materials and Methods

The 23 Willow Warbler, 21 Reed Warbler and 12 Blackcap nests described in Chapter 2 were investigated to determine the effect of air movement on the thermal properties of nests. Thermal properties of the nests were tested under three conditions: 1) still-air in the laboratory; 2) still-air within a wind tunnel; and 3) moving air within a wind tunnel. Two Blackcap nests were excluded from laboratory still air trials because they were unavailable for use due to date of arrival at the University of Lincoln whilst the experiment was conducted (see Appendix 3.1).

The investigations followed the methodology of Deeming and Gray (2016a) to measure the thermal properties of the nests using *i*Button® temperature loggers (Maxim Integrated, San Jose, CA). All temperature loggers were programmed to record temperature data (ºC) every minute. To raise their temperature, temperature loggers were heated to 80ºC by immersion in a water bath (Mainwaring *et al.* 2012, Smith *et al.* 2015, Deeming and Gray 2016a, Gray and Deeming 2017). At 80ºC , temperature loggers were removed from the water bath and excess water was removed with a paper towel.

For all trials, two polystyrene columns were constructed on the basis of the depth of an individual nest's cup that would allow the temperature logger to make contact with the inside surface of the cup of the inverted nest. One heated temperature logger was placed on top of a polystyrene column and the inverted nest was placed on top with its rim resting on a polystyrene base (30 mm thick). The second heated temperature logger was placed on the second polystyrene plinth within 10 cm of the nest at the same height as the first temperature logger (Figure 3.1). A third temperature logger was placed in the laboratory or within the wind

tunnel to record room temperature data during thermal trials. Trials were repeated three times for each nest with an interval period of 20 minutes in order for the temperature loggers to reheat in the water bath and for the nests to return to room temperature (Deeming and Biddle 2015).

The first trials were carried out in an open laboratory (measuring 5 x 6 x 4 metres) as described by Deeming and Gray (2016a). Following the protocol described by Gray and Deeming (2017) a wind tunnel (Figure 3.2) was used to provide an environment under still-air in a restricted volume and then under moving air conditions.

Inside the chamber of the wind tunnel, each nest was placed on a polystyrene sheet (33 mm thick) attached to a wooden stand that stood in the base centre of the test chamber to reduce air resistance and minimise restriction of air flow through the tunnel (see Figure 3.3; Gray and Deeming 2017). The height of the stand was adjusted to allow ±5 cm between the centre of the source of moving air and the centre of the nest (Gray and Deeming 2017). A temperature logger fixed to the internal side of the lid recorded air temperature during trials. Nest thermal properties were recorded firstly in still air (tunnel fan turned off) and then in moving air (tunnel fan turned on and set to a maximum of 1.5 $m \cdot s^{-1}$). This one speed produced uniformity between trials and allowed direct comparison of data with that reported by Gray and Deeming (2017).

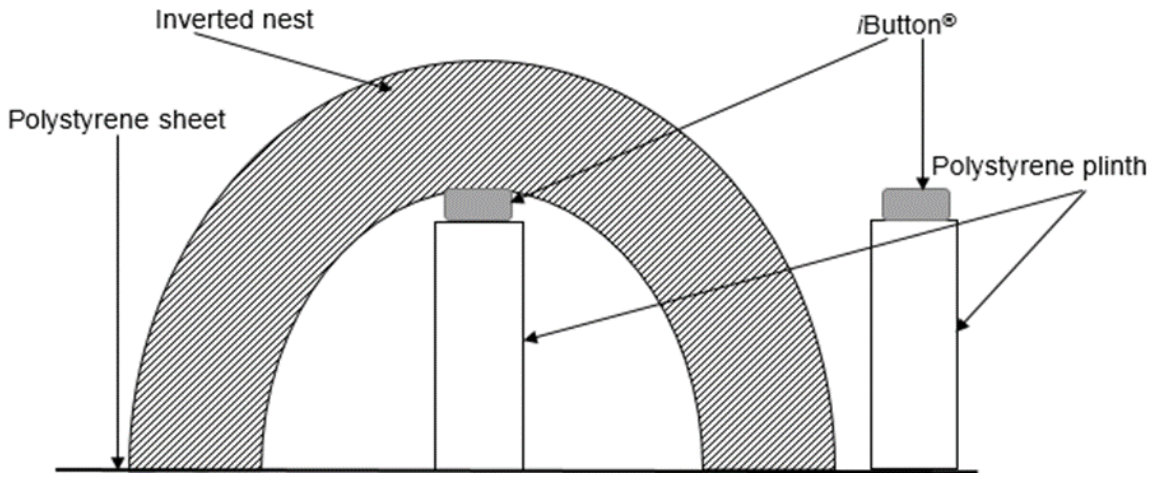

Figure 3.1. Experimental design used to measure the temperature data for thermal measures of passerines nests, adapted from Deeming & Gray (2016).

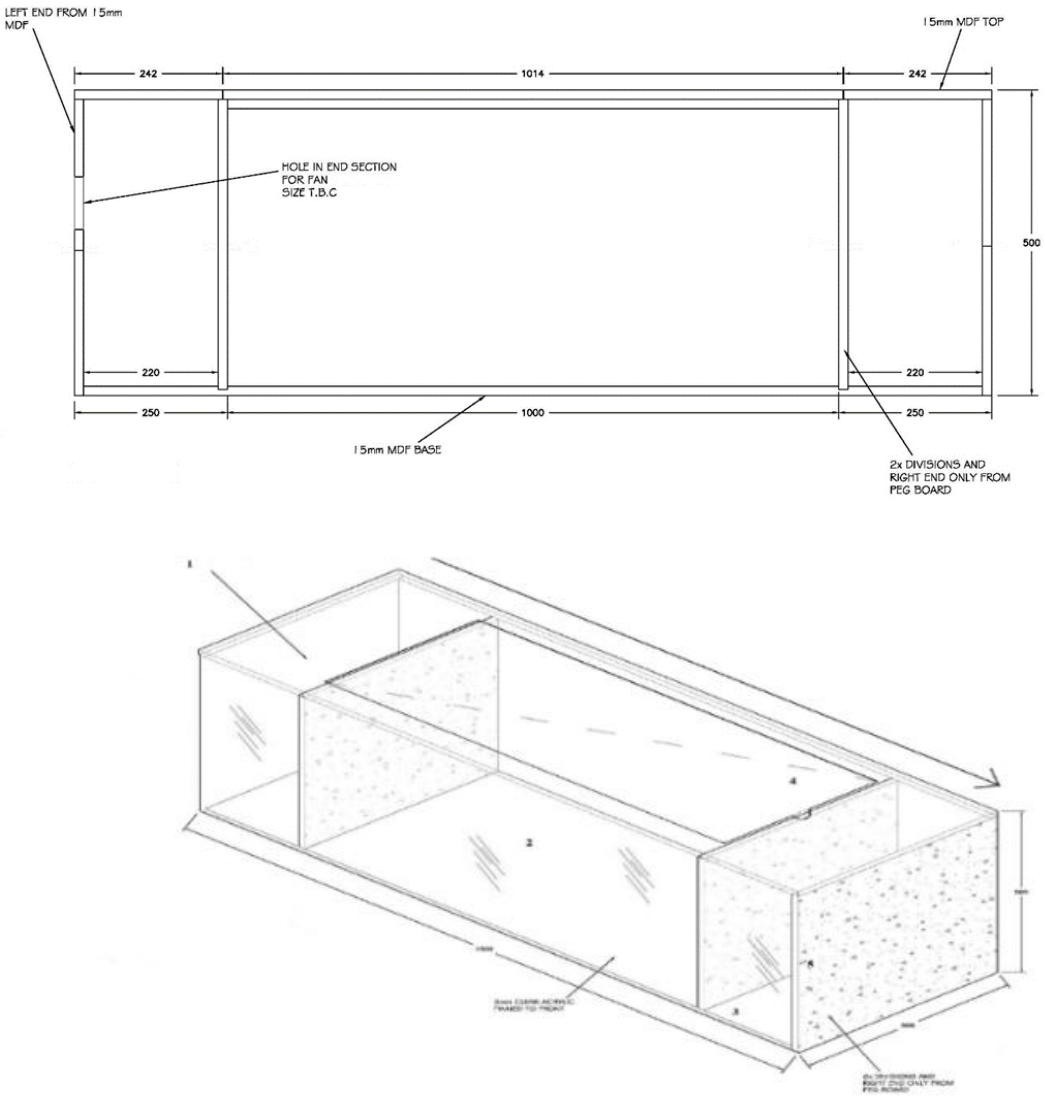

Figure 3.2 Illustrations of the lateral (top) and 3D (bottom) perspectives of the wind tunnel. Arrow indicates the direction of air movement when the fan was operative. Illustrations reproduced from Gray and Deeming (2017).

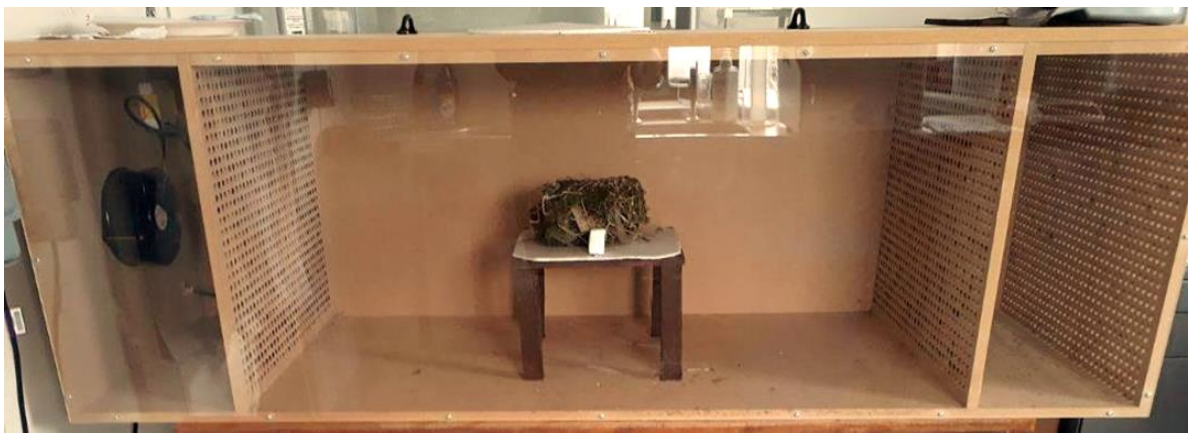

Figure 3.3. The wind tunnel used for thermal experiments with nest *in situ*. Moving from left to right: Fan chamber, test chamber (including trial investigating thermal properties of nests) and settling chamber, respectively.

To determine cooling rates (°C·60s^{-1}), temperature data (°C) collected from the temperature loggers were fitted to logistic models (see McGowan *et al.* 2004, Mainwaring *et al.* 2012 and Deeming and Gray 2016a). The insulatory value (°C·20s^{-1}) of each nest was determined by calculating the difference in cooling rates between the nest and control temperature loggers and dividing by 3 as suggested by McGowan *et al.* (2004) and Mainwaring *et al.* (2012). A large positive insulatory value was indicative of a well-insulated nest (McGowan *et al*. 2004). Internal cooling rates were multiplied by -1 to give positive values. Large positive values of internal cooling rates indicated greater rates of cooling inside the nest.

All statistical analyses were conducted with Minitab (version 17). Analysis of Variance (ANOVA) was used to identify species differences in insulatory values (°C·20s^{-1}) and internal cooling rate (°C·60s^{-1}), in both still and moving air conditions. ANOVA was used to investigate interspecific variation in the difference in insulatory values (°C·20s^{-1}) between still air and moving air, which was also repeated for internal cooling rates (°C·60s^{-1}). General linear mixed model (GLMM) with normal error structure analyses were used to investigate the effect of species and treatment on insulatory values and cooling rates produced for nests. Interaction terms were removed from the model when non-significant. *Post-Hoc* Tukey's HSD was used on statistically significant outputs (P<0.05). Nest ID was included in each model as a random factor to control for the related, non-independent nature of the values.

Spearman signed-rank correlation tests were used to determine relationships between data for thermal properties for all three species combined with nest mass (g), wall thickness (mm) and base thickness (mm) reported in Chapter 2. These dimensions were considered to best reflect the characteristics of the nest that could affect insulation.

For a subset of nests used in thermal experiments, i.e. 12 Blackcap, 10 Reed Warbler and 8 Willow Warbler nests (see Appendix, Chapter 2), Spearman's rank analyses were conducted on thermal properties and material data. Materials that were not present in nests of all species were excluded for Analysis of covariance (ANCOVA) was used to test the effect of species on thermal values whilst controlling for nest dimensions, or mass of nest material, as a covariate. Data were tested for normality using Kolmogorov-Smirnov test and Log-transformed where appropriate.

3.3. Results

Mean values for insulatory values and cooling rates inside nests are shown in Figure 3.4 and 3.5 respectively (see also Table 3.1). With the exception of Blackcap nests values for insulatory values measured in still air were higher in the tunnel than in the laboratory although, the GLMM showed that this was not a significant effect (Table 3.2). By contrast, species was a highly significant factor determining insulatory value (Table 3.2) with Blackcap nests consistently having the highest insulatory values (Table 3.1).

Insulatory values measured in moving air treatment for all species were an order of magnitude greater than for still-air in the tunnel and unsurprisingly this was a highly significant factor that overwhelmed the possible effect of other factors such as variation in nest size and construction (Table 3.3). Willow Warbler nests had the highest insulatory values, with Blackcaps having the lowest, and also produced the largest increase in insulatory value between treatments (Figure 3.4, Table 3.1). There was no significant difference between species in terms of insulatory values produced in both moving air ($F_{2.53} = 0.70$, $P = 0.502$, $r^2 = 0.026$) and still air in the wind tunnel ($F_{2,53} = 0.83$, $P = 0.443$, $r^2 = 0.030$). There was also no significant interspecific variation in the difference in insulatory rate between still air and moving air ($F_{2,53} = 1.34$, $P = 0.270$, $r^2 = 0.048$).

Mean internal cooling rates ($ºC \cdot 60s^{-1}$) were consistently lower in still air conditions in the laboratory and highest under moving air conditions with Blackcap nests having the highest values (Table 3.1; Figure 3.5). Comparison of the two still air conditions showed a significant effect of both species and treatment but there was no significant interaction term (Table 3.2). Comparison of moving air and still air treatments in the tunnel showed highly significant effects of species, treatment and there was a highly significant interaction (Table 3.3). The difference in internal cooling rate between still air and moving air was significantly greater for Blackcap nests ($F_{2, 53} = 11.9$, $P < 0.001$, $r^2 = 0.312$). Blackcap nests produced significantly greater cooling rates in moving air ($F_{2,53} = 23.6$, $P < 0.001$, $r^2 = 0.471$) and still air ($F_{2,53} = 13.9$, $P < 0.001$, $r^2 = 0.344$) treatments than Willow Warbler and Reed Warbler nests. Post-hoc Tukey's HSD revealed there was no significant difference in internal cooling rates produced by Reed Warblers and Willow Warblers.

Table 3.1. Mean (± SD) insulatory values, cooling rates and increase in cooling rate for warbler nests in still air and moving air conditions.

	Willow Warbler N=23	Reed Warbler N=21	Blackcap N=12
Insulatory Value (°C·20s^{-1})			
Still air - laboratory	0.012 ± 0.004	0.015 ± 0.003	0.030 ± 0.025
Still air - tunnel	0.019 ± 0.029	0.017 ± 0.011	0.027 ± 0.016
Moving air - tunnel	0.138 ± 0.030	0.132 ± 0.032	0.121 ± 0.059
Difference: still air in laboratory *vs* still air in tunnel	0.007 ± 0.029	0.002 ± 0.012	0.003 ± 0.035
Difference still air *vs* moving air in tunnel	0.119 ± 0.044	0.115 ± 0.035	0.095 ± 0.051
Cooling rate (°C·60s^{-1})			
Still air - laboratory	0.119 ± 0.012	0.111 ± 0.007	0.139 ± 0.235
Still air - tunnel	0.133 ± 0.012	0.137 ± 0.012	0.175 ± 0.046
Moving air	0.259 ± 0.061	0.233 ± 0.041	0.375 ± 0.077
Difference: still air in laboratory *vs* still air in tunnel	0.014 ± 0.011	0.026 ± 0.011	0.036 ± 0.061
% Increase in cooling rate in still air in laboratory and tunnel	89.4 ± 8.29	81.4 ± 6.97	85.5 ± 28.1
Difference: still air *vs* moving air in tunnel	0.126 ± 0.058	0.096 ± 0.039	0.200 ± 0.086
% Increase in cooling rate in still and moving air in tunnel	194.3 ± 42.3	170.3 ± 28.9	225.1 ± 65.7

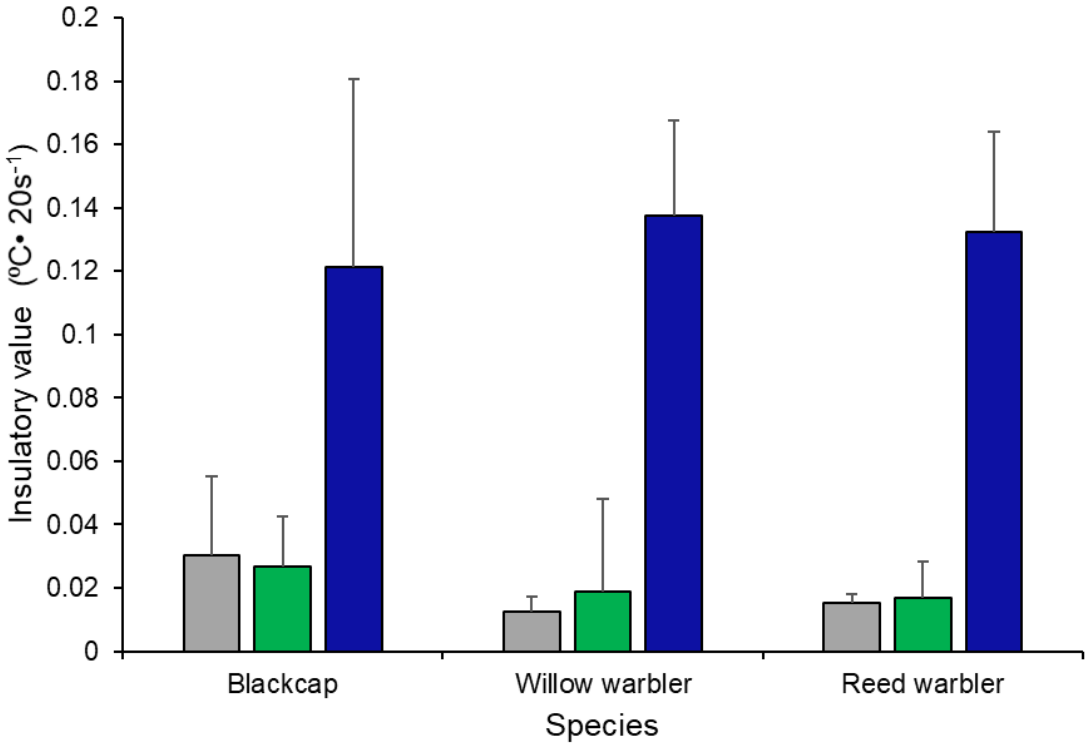

Figure 3.4. Mean (+SD) insulatory values ($^0C \circ 20s^{-1}$) of Sylviidae nests in: 1) still air in the laboratory (grey columns); 2) still air in the wind tunnel (green columns), and 3) moving air (blue columns). N=12 Blackcap, 23 Willow Warbler, and 21 Reed Warbler nests for wind tunnel trials. Only 10 Blackcap nests were used in the laboratory trial (see text).

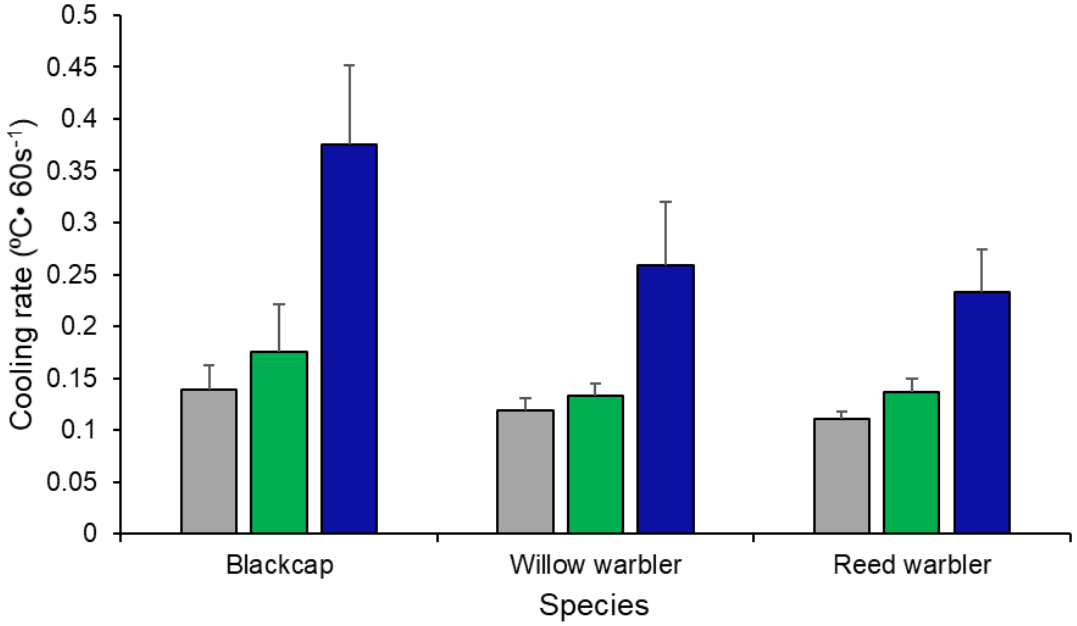

Figure 3.5. Mean (+SD) cooling rate ($^0C \circ 60s^{-1}$) of temperature loggers during three experimental treatments: 1) still air in the laboratory (grey columns); 2) still air in the wind tunnel (green columns), and 3) moving air (blue columns). N= 56 (23 Willow Warbler, 21 Reed Warbler, 12 Blackcap) with the exception of laboratory treatment where only 10 Blackcap nests were available.

Table 3.2. Effect of species and treatment (laboratory conditions and wind tunnel: still air) on thermal measures. Species and treatment assigned as fixed factors, with individual nests modelled as random factors nested within species.

Factor (DF)	Insulatory value ($ºC \cdot 20s^{-1}$) F-value (p-value)	Cooling rate ($ºC \cdot 60s^{-1}$) F-value (p-value)
Species (2,51)	5.9 (0.005)	25.1 (<0.001)
Treatment (1,51)	0.46 (0.502)	39.9 (<0.001)
Interaction (2,51)	0.38 (0.688)	2.3 (0.110)
Nest ID (51,51)	0.83 (0.743)	0.88 (0.678)
R^2	0.514	0.719

Table 3.3. Summary of a General Linear Model (GLMM) for thermal measures with treatment (air movement) and species as a fixed factor. Individual nests were random factors nested within species.

Factor (DF)	Insulatory value ($ºC \cdot 20s^{-1}$) F-value (p-value)	Cooling rate ($ºC \cdot 60s^{-1}$) F-value (p-value)
Species (2,53)	0.2 (0.819)	30.2 (0.001)
Treatment (1,53)	346.5 (<0.001)	293.7 (<0.001)
Interaction (2,53)	1.34 (0.270)	11.9 (<0.001)
Nest ID (53,53)	1.17 (0.287)	1.30 (0.173)
R^2	0.897	0.894

Table 3.4. Spearman Rank correlations for associations between thermal measures and nest dimensions of 56 warbler nests. Rho values with *P*-values in parentheses, DF= 54.

	Nest Dimensions		
Thermal properties	Nest mass (g)	Wall thickness (mm)	Base thickness (mm)
Insulatory value: still air	-0.062 (0.652)	-0.050 (0.715)	0.142 (0.295)
Insulatory value: moving air	0.013 (0.339)	-0.024 (0.859)	0.031 (0.819)
Difference in insulatory value	0.187 (0.167)	0.063 (0.642)	0.027 (0.846)
Cooling rate: still air	0.585 (<0.001)	0.162 (0.234)	0.474 (<0.001)
Cooling rate: moving air	0.294 (0.028)	-0.131 (0.337)	0.398 (0.002)
Difference in cooling rate	-0.089 (0.514)	0.156 (0.252)	-0.224 (0.097)

Table 3.5. Results of analysis of covariance (ANCOVA) for internal cooling rate to test the effect of species as a fixed factor and specified nest dimension (nest mass (g) or base thickness (mm)) as a covariate.

Cooling rate (ºC◦60s^{-1}):	Species (2,50)	Log_{10} Nest mass (1,50)	r^2
Moving air	17.3 (<0.001)	0.00 (0.972)	0.471
Still air	5.01 (0.010)	4.19 (0.046)	0.393
Cooling rate (ºC◦60s^{-1}):	Species (2,50)	Log_{10} Base thickness (1,50)	r^2
Moving air	15.9 (<0.001)	0.47 (0.498)	0.476
Still air	6.97 (0.002)	4.33 (0.042)	0.394

There were no significant correlations between insulatory values, in either still or moving air, or the difference in insulatory values between these treatments with either nest mass, wall or base thickness (Table 3.4). Nest mass was significantly negatively correlated with internal cooling rates produced by Warbler nests in both still air and moving air treatments but the difference in cooling rates did not correlate (Table 3.4). Wall thickness did not significantly correlate with internal cooling rate in any way (Table 3.4). By contrast, base thickness had a significant negative correlation with internal cooling rate in both still air and moving air (Table 3.4).

Log_{10} nest mass was a significant covariate and species was a significant factor influencing internal cooling rates in still air (Table 3.5). By contrast, Log_{10} nest mass was not a significant covariate where species was a significant factor, on the internal cooling rates in moving air conditions (Table 3.5). Furthermore, Log_{10} base thickness of nests had a significant effect on internal cooling rates in still air where species was a significant factor on internal cooling rates in still air (Table 3.5). Log_{10} base thickness was not a significant covariate on cooling rates produced for nests in moving air, however species was a significant factor (Table 3.5).

For the subset of nests where nest composition was recorded insulatory values measured in still air in the tunnel had significant negative correlations with masses of moss and feather mass, and significant positive correlations with the masses of silk and artificial materials (Table 3.6). By contrast, in moving air mass of lichen showed a significant negative correlation with insulatory value whereas mass of silk had a significant positive correlation (Table 3.6). The difference in insulatory value between moving air and still air showed a significant negative correlation with the amount of lichen. All other relationships were non-significant (Table 3.6).

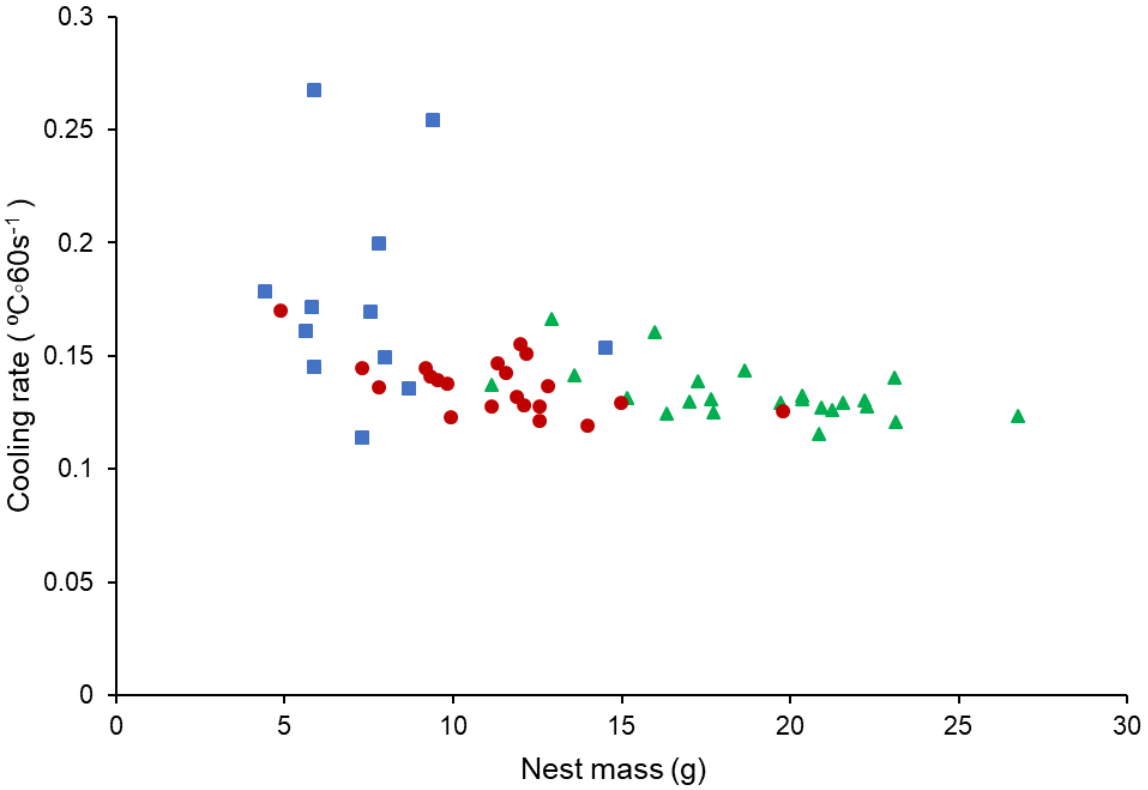

Figure 3.6. Effect of nest mass (g) on the internal cooling rates (^{0}C◦ 60s^{-1}) of warbler nests produced in still air conditions. Blue square = Blackcap; green triangle = Willow Warbler; and red circle = Reed Warbler.

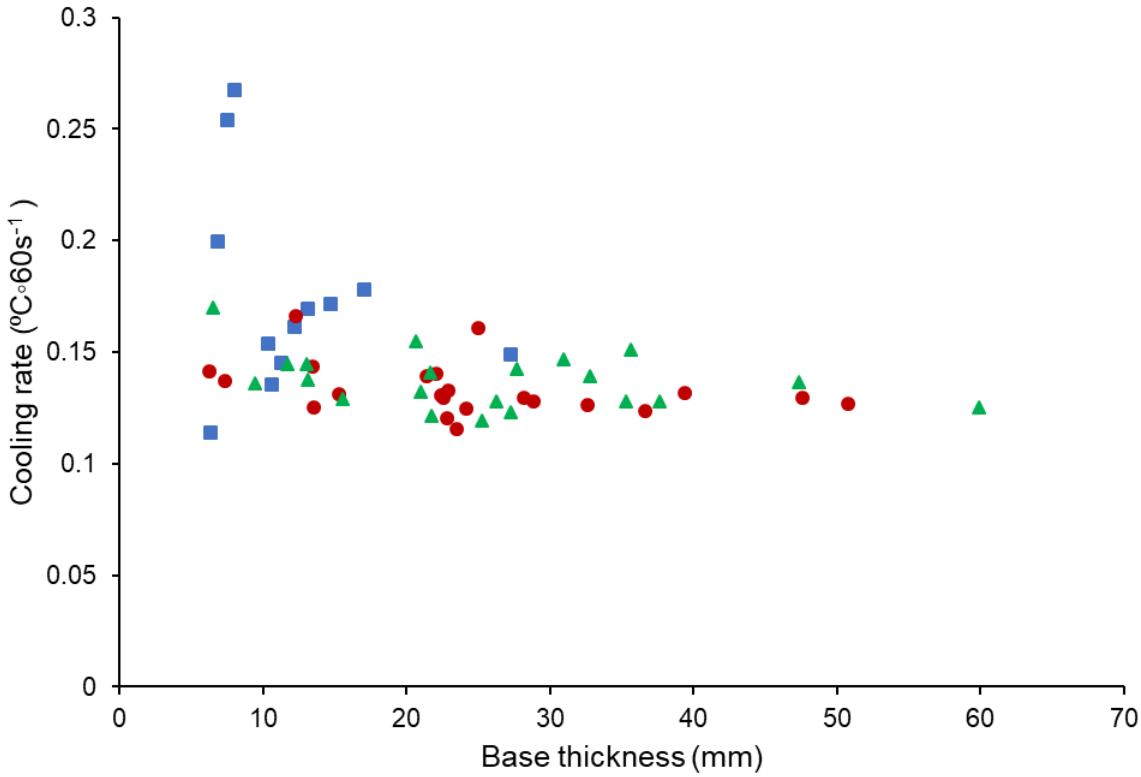

Figure 3.7. Relationship between nest base thickness (mm) and internal cooling rates (^{0}C◦ 60s^{-1}) of 56 warbler nests in still air conditions. Blue square = Blackcap; green triangle = Willow Warbler; and red circle = Reed Warbler.

Table 3.6. Spearman signed rank correlation between thermal properties of nests (insulatory values ºC•20s^{-1}; cooling rate ºC•60s^{-1}) and nest materials for a subset of 30 nests for species combined. Rho values with *P*-value in parentheses. Shaded cells indicate significant correlations.

	Insulatory value: still air	Insulatory value: moving air	Difference in insulatory value	Cooling rate: still air	Cooling rate: moving air	Difference in cooling rate
Grass	-0.136 (0.743)	0.086 (0.653)	0.204 (0.278)	-0.488 (0.006)	-0.682 (<0.001)	-0.621 (<0.001)
Moss	-0.518 (0.003)	-0.136 (0.475)	-0.018 (0.924)	-0.135 (0.477)	-0.063 (0.739)	0.014 (0.942)
Hair	-0.145 (0.444)	0.172 (0.363)	0.202 (0.285)	-0.139 (0.463)	-0.026 (0.892)	0.040 (0.836)
Feather	-0.556 (0.001)	0.114 (0.548)	0.292 (0.117)	-0.105 (0.582)	-0.142 (0.453)	-0.063 (0.743)
Leaves	0.135 (0.477)	0.100 (0.599)	0.006 (0.977)	0.112 (0.555)	0.319 (0.086)	0.317 (0.088)
Roots	-0.096 (0.614)	-0.149 (0.431)	-0.134 (0.481)	0.264 (0.158)	0.340 (0.066)	0.257 (0.170)

Table 3.7. Analysis of covariance results for thermal measures with species as a fixed factor and nest material as a covariate. *F*- values with *P*-values in parentheses.

	Covariate	Species	Material	r^2
Insulatory value	DF	(2,26)	(1,26)	
Still air (^{0}C ◦ 20s^{-1})	**Log_{10} (Moss)**	1.51 (0.239)	0.69 (0.415)	0.264
Still air (^{0}C◦ 20s^{-1})	**Log_{10} (Feather)**	2.01 (0.155)	0.44 (0.514)	0.257
Internal cooling rate				
Still air (^{0}C ◦ 60s^{-1})	**Log_{10} (Grass)**	3.20 (0.057)	0.05 (0.823)	0.308
Moving air (^{0}C ◦ 60s^{-1})	**Log_{10} (Grass)**	4.21 (0.026)	5.85 (0.023)	0.672
Difference in thermal measures				
Internal cooling rate (^{0}C◦60s^{-1})	**Log_{10} (Grass)**	1.13 (0.340)	5.76 (0.024)	0.527

Analysis of covariance did not show that species was a significant factor nor was the Log_{10}-transformed material mass for insulatory values measured either still air or moving air (Table 3.7).

The mass of grass present in the nest showed significant negative correlations with internal cooling rate in still air (Table 3.6). Similar patterns were seen for moving air cooling rates and significant positive relationships were seen with the mass of stems (Table 3.6). The difference in cooling rate between the two treatments showed positive correlations with stems and a negative relationship with the amount of grass (Figure 3.9; Table 3.6). Species was a significant factor for internal cooling rate for grass in moving air, and for grass in still air. Species was a significant factor when examining the difference in internal cooling rates when controlling for stems as a covariate (Table 3.7). Grass was a significant covariate influencing cooling rates in moving air where species was a significant factor. By contrast, species was not a significant factor influencing the difference in cooling rates where grass was a significant covariate (Table 3.7).

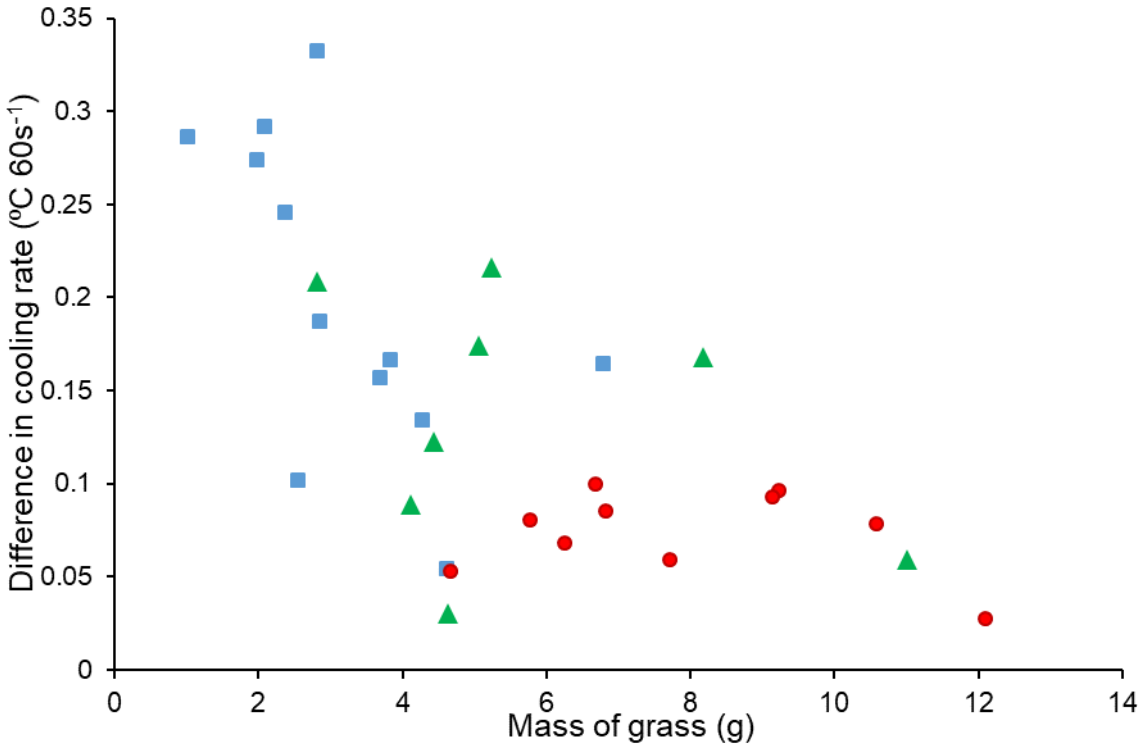

Figure 3.8. Relationship between mass of grass and the difference in internal cooling rates ($^0C\circ\ 60s^{-1}$) for 30 warbler nests. Blue square = Blackcap; green triangle = Willow Warbler; and red circle = Reed Warbler.

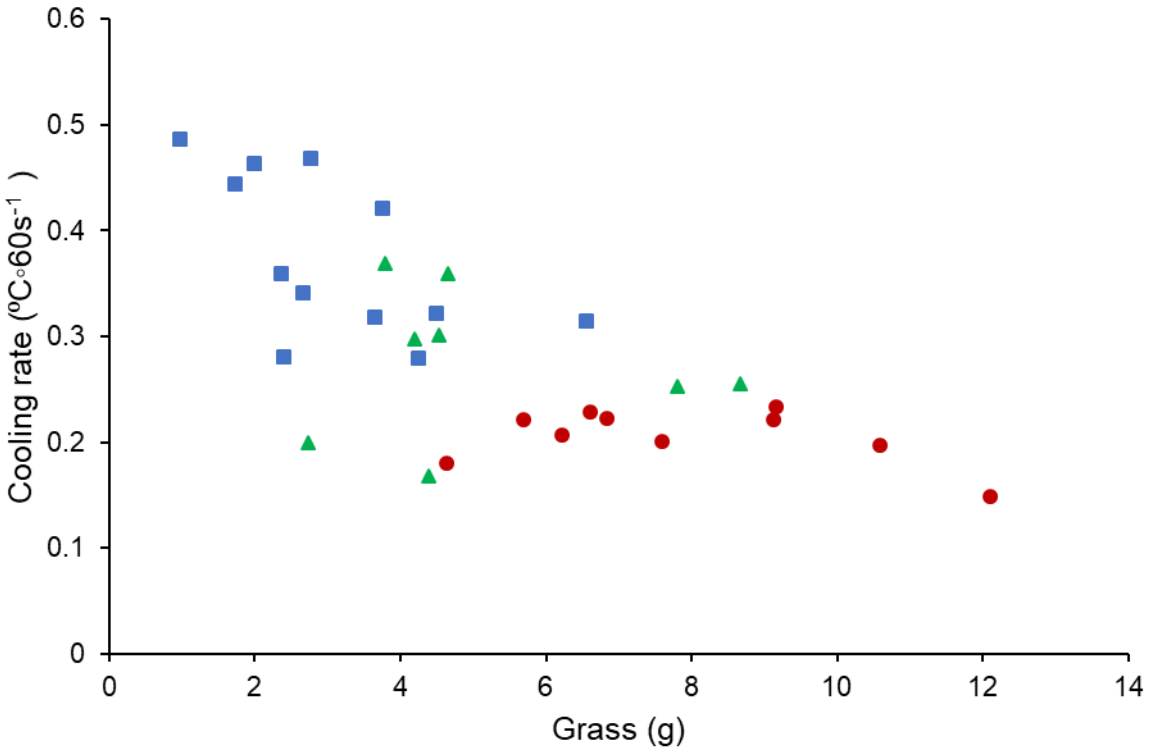

Figure 3.9. Relationship between the mass of grass (g) in 30 warbler nests and the internal cooling rates produced during moving air conditions. Blue square = Blackcap; green triangle = Willow Warbler; and red circle = Reed Warbler.

3.4. Discussion

Insulatory values of warbler nests did not correlate with nest dimensions but did correlate with a few materials in the nest. Internal cooling rates for Warbler nests were influenced by certain nest dimensions and nest materials. When exposed to moving air, Warbler nest insulatory values increased by an order of magnitude although internal cooling rates also increased. Species differences in thermal properties were seen for insulatory values and cooling rates in still air conditions. Despite this, there was no significant interspecific variation in insulatory values of Warbler nests when exposed to air movement.

3.4.1. Comparison of IV results with previous reports

Published values for insulatory values range from 0.015 (^{0}C◦20 s^{-1}) in Goldfinches *Carduelis carduelis* (Gray and Deeming 2017) through to 0.062 (^{0}C◦20 s^{-1}) for the Common Redstart *Phoenicurus phoenicurus* (Deeming and Mainwaring 2015). Insulatory values for Willow Warbler and Reed Warbler nests were at the lower end of this range whereas Blackcap nests were more comparable to those reported for Great Tit nests (see Deeming and Mainwaring 2015) and were similar in magnitude to those recorded for other passerines (Deeming and Mainwaring 2015, Gray and Deeming 2017).

Gray and Deeming (2016a) reported that insulatory values exhibited a negative correlation with attentive patterns during incubation but no relationship with bird mass. Attentiveness of the uniparental species, Willow Warblers and Reed Warblers, fit generally on the plot by Deeming and Gray (2016a). Reported attentiveness of Willow Warblers is 74.3%, and 68.9% for Reed Warblers (Cramp 1992). Blackcaps display biparental continuous care, with the male incubating 45% and the female incubating 55% (Cramp 1992).

Average nest insulatory values for each warbler species increased dramatically when in moving air conditions compared with still air. Gray and Deeming (2017) also showed a ten-fold increase in nest insulatory values for several species from Fringillidae and Motacillidae passerines. Although the insulatory values increase for nests exposed to air movement, the nest is not gaining insulation in absolute terms, which is confirmed by increased internal cooling rates during air movement. Internal cooling rates are greater in moving air than in still air, suggesting that air movement does indeed alter the internal

microclimate of the nest. Insulatory values represent the relative difference in the change in the cooling rate of temperature loggers inside the nest and the temperature loggers outside of the nest. Therefore, the greater insulatory values during periods of air movement reflect the nest acting as a buffer to mitigate the effect of moving air on nest microclimate.

Mean insulatory values for warbler nests in still air were not significantly influenced by location of trials. The internal cooling rates produced by warbler nests in still air conditions were significantly affected by the location of trials. Contrary to previous studies, within the open laboratory used here, internal cooling rates of nests were lower than those produced in the closed wind tunnel (Deeming and Gray 2016a; Gray and Deeming 2017). Internal cooling rates may have been reduced by room temperature fluctuations and heat radiation from conducting trials in a laboratory with direct sunlight. The difference in thermal measures between locations is minimal in comparison to the difference in thermal measures between still air and moving air trials. Nonetheless, there is an effect of trial location on the results produced for nest thermal properties thus conducting trials in a controlled, closed chamber may be beneficial to maintaining accuracy and repeatability. Conducting still air trials before the moving air trials may also prevent material loss or creation of holes which perhaps alter internal cooling rates.

3.4.2. Effects of air movement on internal cooling rates

Gray and Deeming (2017) reported an increase in both cooling rate and insulatory values for Motacillidae and Fringillidae nests when exposed to moving air. Nests have also shown to reduce rate of cooling of steel balls in the nest cup in comparison to nest absence (Ar and Sidis 2002). Warbler nests produced similar percentage increases in rate of heat loss to those reported for other passerines in the literature (Palmgren and Palmgren 1939, Heenan and Seymour 2012, Gray and Deeming 2017). Palmgren and Palmgren (1939) reported an increase in cooling rates of water of 144% when placed inside Common Rosefinch *Carpodacus erythinus* nests and 191% for Chaffinch nests during windy conditions in comparison to still air conditions. Under laboratory conditions, thermal conductance increased by 170% at maximum air speed of 0.88 ms^{-1} in passerine nests investigated by Heenan and Seymour (2012). Maximum speed

of air movement used here was almost double that of Heenan and Seymour (2012) however the lowest average percentage increase achieved was 170% and maximum percentage increase was at 225%. Warbler nests may be more insulating than the nests of the two passerine species studied by Heenan and Seymour (2012) as the cooling rates reported here were not double that of those reported for nests exposed to half the air speed. Percentage increase in internal cooling rate was overall greater for warbler nests than for nests reported by Gray and Deeming (2017). The wind tunnel and air speed (1.5 ms^{-1}) used here was identical to that used by Gray and Deeming (2017), but average percentage increase of cooling rate was 156% for Motacillidae and Fringillidae nests combined, compared to 190% reported here. Maximum percentage increase reported here was 225% however internal cooling rates increased by maximum 188% for nests investigated in Gray and Deeming (2017). This may be indicative of Warbler nests providing poorer insulation to the clutch or the nests may be more vulnerable to climatic changes than nests of previously reported families.

3.4.3. Effect of mass and dimensions on thermal measures

The results presented here supported the hypothesis that the thermal properties of warbler nests are influenced by nest mass. Akresh *et al.* (2017) reported a negative effect of nest mass on cooling rates of Prairie Warbler nests, suggesting that heavier nests were better insulated. Gray and Deeming (2017) also found a significant, positive effect of nest mass on the thermal properties of nests from several passerine species. Similar to Motacillidae and Fringillidae species investigated by Gray and Deeming (2017), warbler nest internal microclimate was influenced by nest mass. Nests from different species but with the same nest mass produced similar insulatory values. For example, Gray and Deeming (2017) reported Linnet nest mass at 18.3g, which is similar to an average nest mass of 18.9 g for the Willow Warbler. Linnet nest insulatory values averaged 0.018 ($^0C \circ 20\ s^{-1}$) and 0.134 ($^0C \circ 20\ s^{-1}$) in still air and moving air, respectively. Equivalent values for Willow Warbler nests were 0.019 ($^0C \circ 20\ s^{-1}$) and 0.138 ($^0C \circ 20\ s^{-1}$). Average Meadow Pipit nest mass (12.4g) reported in Gray and Deeming (2017) was similar to Reed Warbler nest mass (11.3g) reported here and their thermal measures were almost identical. For example, Reed

Warbler and Meadow Pipit nests mean insulatory value in still air was 0.017 (^{0}C◦20 s^{-1}). During periods of moving air, insulatory values for Meadow Pipit nests and Reed Warbler nests were 0.134 (^{0}C◦20 s^{-1}) and 0.132 (^{0}C◦20 s^{-1}) respectively (Gray and Deeming 2017). Interal cooling rates in moving air conditions for Reed Warbler and Pipit nests were 0.233 (^{0}C◦60 s^{-1}) and 0.234 (^{0}C◦60 s^{-1}) respectively (Gray and Deeming 2017). Blackcap nests were lighter in mass than those nests reported in Gray and Deeming (2017), and whilst internal cooling rates were higher for Blackcap nests their insulatory values were not dissimilar to Goldfinch and Chaffinch nests (Gray and Deeming 2017).

Previous studies have shown wall thickness influences nest thermal properties (Heenan and Seymour 2012; Akresh *et al*. 2017), but wall thickness was not associated with the thermal properties of warbler nests in this study. For all species here, nest base thickness was a factor influencing the internal cooling rate of temperature loggers inside the nest. A thicker nest base may allow for improved convection around the nest and provide greater insulation, resulting in reduced heat loss from the cup. Methodology may be a key factor in influencing the effect of nest dimensions on nest thermal properties. Here, data loggers rested on the nest base, whereas in other studies, a heated metal sphere has been used to measure nest thermal properties (Heenan and Seymour 2012). Heat energy is most likely to be lost through the nest base rather than the nest walls in this study, resulting in an effect of base thickness rather than nest wall thickness. Although base thickness was not a factor influencing thermal properties in Fringillidae and Motacillidae nests (Gray and Deeming 2017), species with similar base thickness to warbler nests here produced similar thermal values. For example, Meadow pipits and Willow Warblers have similar average base thickness and insulatory values and produced similar internal cooling rates (Gray and Deeming 2017). Further comparative studies of open-cup passerine nests across multiple species may be useful in understanding interspecific variation in nest construction in relation to insulation.

Although nest wall thickness did not have an effect on the thermal properties of warbler nests, nest wall porosity and density may have influenced the thermal properties produced for warbler nests. White Crowned Sparrow *Zonotrichia leucophrys* nests with low porosity lost heat at a slower rate than

nests with highly porous walls (Kern 1984). Blackcap nests have loosely constructed nest walls and base with visible gaps in the material and unsurprisingly experienced the greatest rate of heat loss in all test conditions, most likely due to heat energy moving through the gaps in the nest. Willow Warbler nests produced the slowest cooling rates during still air trials inside the wind tunnel, suggesting that Willow Warbler nests had the best insulation. During episodes of air movement, however, temperature loggers cooled at the slowest rate when inside Reed Warbler nests. Reed Warbler nests are tightly woven, dense cups with no visible gaps, whereas the materials used to construct Willow Warbler nests were easily malleable in comparison. The tightly woven nest walls are likely to be less porous than Willow Warbler nests walls, resulting in a lower rate of convective heat loss from Reed Warbler nests when exposed to moving air. Directly measuring structural wall porosity may prove useful in further understanding nest construction in relation to their thermal properties. Researchers should also consider investigating variation in wind speed and structural wall porosity of birds' nests at the nest site to allow insight into the thermal properties of nests in their natural location.

Nest site may also influence nest thermal properties and nest construction. Willow Warblers are ground nesting species, whereas Reed Warblers are elevated, thus should experience more convective cooling than Willow Warblers. Previous studies have recorded similar thermal values for ground nesting species (Gray and Deeming 2017) as those recorded for Willow Warblers here. Meadow Pipits have a lighter nest mass than Willow Warbler nests (12.44 g, Gray and Deeming 2017) however both produce similar average insulatory values in still and moving air conditions. Typically, ground nests are subjected to better vegetative cover and are exposed to lower velocities of air movement than elevated nests (With and Webb 1993; Kern 1984). The combination of lower wind velocity and being under vegetative cover decreases the convective heat loss from the nest (With and Webb 1993). Therefore, Reed Warbler nests are likely to be better adapted at mitigating the effect of air movement on the internal microclimate of the nest than Willow Warblers due to the difference in nest site. The reduced rate of heat loss by Reed Warbler nests may therefore be a reflection of nest site location and constructing a dense nest with low porosity in response to the climate at the nest site.

Blackcap nests are also elevated in comparison to Willow Warblers but have highly porous nests and a high rate of cooling. Where previous *in situ* studies have found egg temperatures to be lower and less stable in elevated nests than in ground nests, *ex situ* studies have found that elevated nests are typically better insulated than ground nests under laboratory conditions (Zebra and Morton 1983, Kern 1984). Although Blackcap nests are the least insulating during *ex situ* studies, they may be constructed for optimal nest temperature for chick rearing and incubation of the clutch at the nest site. Blackcaps typically nest in shrubs and bushes that provide vegetative cover (Cramp 1992, Weidinger 2002) therefore may experience shelter from prevailing winds. Nevertheless, Blackcap nests still act as a buffer from air movement.

Air within the nest walls may act as a good insulator. Air gaps within the nest structure have been reported to provide insulation for some species (Deeming and Biddle 2015). Trapped air within the walls of Blackcap nests may explain the two-fold reduction in cooling rate in still air compared to moving air under still air conditions. Air gaps may provide insulation however they may not useful in mitigating the effect of air movement on convective heat loss (Deeming and Biddle 2015). Trapped air within the gaps in material may also explain why Blackcap nests have similar insulatory values during periods of air movement as Reed Warbler and Willow Warbler nests despite containing less nest material. Air gaps in Reed Warbler and Willow Warbler nests material may also provide additional insulation. Further investigation into the role of air-gaps in passerine nests may therefore aid understanding of nest thermal properties.

3.4.4. Effects of materials on thermal measures

Animal derived materials, such as feathers and hair present in Warbler nests did not have a significant effect on the thermal properties of the nest. A non-significant effect of feathers on warbler nest insulation contradicts previous findings for the effect of feathers on some passerine nest thermal properties (Hilton *et al.* 2004; McGowan *et al.* 2004). Feathers are a common material found in passerine nests (Collias and Collias 1984; Pinowski *et al.* 2006) and have been associated with providing thermal benefits to both offspring and parent birds (Møller 1987; Lombardo *et al.* 1995; Hilton *et al.* 2004; Windsor *et al.* 2013). In Tree Swallows *Tachycineta bicolor,* chicks reared in nests with feathers had

greater body mass, greater flight feather length and longer tarsi length than those reared in nests with feathers experimentally removed (Lombardo *et al.* 1995; Dawson *et al.* 2011). In warblers, feather mass contributed to a very small percentage of the total nest mass compared to other passerine species nests. For example, in Long-tailed Tit nests, feathers lining the nest contribute to 41% of nest mass and are the most important nest material for insulation (McGowan *et al.* 2004). Fewer feathers present in the nest may seem disadvantageous to warbler offspring survival as feathers are highly insulating (Hilton *et al.* 2004, see below).

Animal-derived materials are more prevalent in cavity-nesting species than open-nesting species (Deeming and Mainwaring 2015). Cavity nesting species such as Great tits and Blue tits actively select for particular animal derived materials to line the nest cup (Britt and Deeming 2011). Warblers investigated here are open-cup nesting passerines and nest at a relatively low height (Cramp 1992), thus, may be more exposed to predators than cavity nesting species or species that nest at greater heights. Warblers may be actively avoiding feathers in open cup nests may therefore reduce the nest from being conspicuous to predators, presenting a trade-off between insulation and nest predation. Feathers are also hydrophobic; therefore, water may pool in the nest cup if it were to rain on an uncovered nest, undoubtedly affecting the nest microclimate (Hilton *et al*. 2004, Heenan *et al.* 2013). Of the three species investigated here, feathers were present in both greatest mass and greatest proportion in Willow Warbler nests. Willow Warbler nests are typically found on the ground under the cover of shrubs and can be domed (Cramp 1992), therefore addition of feathers may be less conspicuous to predators than if they were present in Blackcap or Reed Warbler nests.

Plant-derived materials dominate the composition of Warbler nests, for example, Reed Warbler nests had the greatest mass of grass and were primarily constructed with dry grass (Chapter 2). Grass was the most common component across the nests of the three species and had a significant influence on the internal cooling rates of warbler nests. Mass of grass significantly influenced internal cooling rates for warbler nests when in moving air and also the difference in cooling rate between still air and moving air. Previous studies have found evidence that grass provides insulation to the nest. Mainwaring *et al.* (2014b)

showed that Common Blackbird nests with greater mass of dried grass were better insulated. The effect of grass as an insulating material may be heightened in moving air due to the increased rate of heat loss. Grass is a common component found in both the cup lining and structural walls in the warbler nests here, therefore heavier nests may have more grass, which could minimise the rate of cooling of the nest. The influence of grass on internal cooling rates is greatest for Blackcap nests (Figure 3.9). Greater grass mass may result in reduced wall and base porosity in Blackcap nests, reducing the internal cooling rate during air movement. Reed Warbler nests are woven tightly with grass and are built primarily with dry grass. The effect of grass mass on Reed Warbler cooling rates was minimal, therefore other unmeasured factors such as nest wall density and porosity may influence the internal cooling rates of Reed Warbler nests. Mass of leaves has been shown to be related to nest insulation in European Robins (Taberner Cerezo and Deeming 2016), however leaves showed no significant correlation with thermal measures in warbler nests. Moss is a major component of Willow Warbler nests, however there was no significant influence of moss mass on nest thermal properties. The high proportion of moss in Willow Warbler nests may therefore contribute to other unmeasured factors, such as water absorption (Wesolowski *et al.* 2002; see Chapter 4).

3.4.5. Conclusions

Nest building, incubation and chick rearing are energetically costly for parent birds (Nord and Williams 2015). Blackcap males and females share incubation equally (Cramp 1992; Weidinger 2002; Redfern 2010), thus energetic costs of Blackcap parents may be less demanding than in the case of a single incubating parent. A large, highly insulating nest may not be needed if the parents are able to share energy expenditure between each other. Building a small nest during nest construction perhaps conserves energy and may also allow for more energy to be spent during incubation and chick rearing (Mainwaring and Hartley 2009, Britt and Deeming 2011). Although heat loss from the nest may seem detrimental to offspring development, allowing heat energy to be removed from the nest, perhaps via air gaps, may also be beneficial in regulating the thermal

environment and preventing heat stress (Deeming 2016; Rodríguez and Barba 2016a, 2016b).

In conclusion, the thermal properties of warbler nests are affected by air movement however nests act as a buffer to mitigate the effect of air movement, an effect which has been seen in other species (Gray and Deeming 2017). The construction of the nest may be adapted in regards to nest materials and nest dimensions in order to reduce the negative effects of increased air movement on the clutch. The potential function of certain nest materials and nest dimensions in the thermal properties of avian nests is still unclear. As only a subset of warbler nests were used for deconstruction and composition analysis, further studies should incorporate the quantification of nest materials, from both open cup and hole-nesting species, in order to understand the role that nest composition may have in achieving successful incubation of the clutch. Considering the influence of nest wall structure and porosity on the thermal properties of passerine nests may prove beneficial in understanding combined effect of nest characteristics on the thermal properties of nests. A standardised method to investigate the thermal properties of nests would be advantageous to interspecific and intraspecific comparisons. It is recommended that researchers incorporate more biologically relevant methods, such as dummy eggs resting in a nest to investigate the thermal conductance of nests. Insulatory value has proved beneficial when testing different environmental conditions however it is limited by its contextual relevance. As suggested in this chapter, ensuring that testing environments are controlled for potential limiting factors, for example in a closed chamber such as the wind tunnel featured here, is vital in producing accurate thermal measures for avian nests.

Chapter 4: Investigating the hydrological properties of Sylviidae nests.

4.1. Introduction

Environmental conditions at the nest site have been shown to affect nest building behaviour, including the choice of specific nest materials, nest dimensions and nest orientation (Kern 1984, Sidis *et al.* 1994, Mainwaring and Hartley 2008, Britt and Deeming 2011, Deeming *et al.* 2012, Mainwaring *et al.* 2012, Mainwaring *et al.* 2014a). Effects of temperature, prevailing winds and solar radiation on nest building behaviour have been considered in previous studies (see chapters 1 and 3), however the effect of precipitation and moisture has rarely been investigated.

Rainfall has been shown to influence parental behaviour and fledgling body condition in cavity nesting passerines (Radford *et al.* 2001, Öberg *et al.* 2015). During the early nestling period altricial chicks, such as Great Tits *Parus major*, are unable to regulate their body temperature above the ambient temperature (Radford *et al.* 2001). In Great Tits, female visitation rates reduced during periods of rain, perhaps to compensate for increased thermoregulatory demands of early nestling chicks (Radford *et al.* 2001). Fledging weight decreased with increasing number of wet hours during the first week of the nestling period (Öberg *et al.* 2015). Extended periods of rain during the nestling stage of Northern Wheatears *Oenanthe oenanthe* was associated with reduced fledging success and produced long-term effects on parental survival (Öberg *et al.* 2015).

The absence of studies investigating the effect of rainfall on nest design is staggering considering that rain is ubiquitous across the globe. The requirement to protect avian offspring from moisture and precipitation may influence avian nest design, including the dimensions of the nest and specific materials used for construction. Exposure to suboptimal humidity and moisture levels during incubation and chick rearing may have an adverse impact on offspring survival. For example, wet nest materials are less insulating than dry materials, therefore clutches exposed to a wet nest are expected to cool at a greater rate than in a dry nest (Hilton *et al.* 2004). Ground-nesting species, such as Willow Warbler,

may be especially susceptible to adverse effects of rainfall due to water pooling underneath the nests and rainfall from above, which may increase chick mortality (Morrison *et al.* 2015). Nest loss due to water-saturated nests, or nest soaking, has been reported in several cavity-nesting passerine species (East and Perrins 1988, Wesolowski and Stawarczyk 1991). Previous studies have suggested that a primary function of a thick, bulky outer nest may be to protect against moisture and nest saturation (Wesolowski *et al.* 2002). Birds may construct nests with specific materials to ensure optimal nest humidity and reduce the chances of nest soaking. In several Australian passerine species, precipitation levels have been shown to influence the selection of nest materials (Heenan *et al.* 2015). Nests built by species in warm climates with heavy rainfall have been shown to use less insulating materials than in a drier climate. These nests may have reduced absorptive properties and the ability to dry out quickly, which may prove beneficial in restoring the optimal nest microclimate (Heenan *et al.* 2015). Slagsvold (1989b) investigated water retention and drying times of nests from 6 European passerine species by submerging nests in water and hanging them to dry. The time taken for nests to return to original dry mass was used to indicate drying times (Slagsvold 1989b). Slagsvold (1989b) suggested that nest materials and nest structure had an effect on water retention. For example, nests that were loosely constructed and made of grass and roots dried out faster than nests with a denser structure. Rowher and Law (2010) also investigated the hydrological properties of nests built by Yellow Warblers *Dendroica petechia* in northerly and southerly populations of North America. Hydrological properties were tested by submerging 10 nests and measuring water absorbed by nests and drying times. Nests built in the more northerly region, Churchill, absorbed more water and took longer to dry than those built in the southern region of Elgin (Rowher and Law 2010).

Most studies investigating the hydrological properties of avian nests are specific to hole nesting species (Snyder *et al.* 1987; Rowley 1990; Wesolowski and Stawarczyk 1991; Wesolowski 1996; Radford *et al*. 2001; Wesolowski *et al*. 2002; Öberg *et al.* 2015). A prominent cause of nest soaking in hole nests is likely to be from tree sap within the cavity rather than direct rainfall (Wesolowski 1996). Open cup nests are likely to be vulnerable to direct rainfall thus elevated risk of nest soaking than hole nests (Collias and Collias 1984). Preventing rainfall

directly entering the nest cup may have a greater influence on nest construction in open cup nests than in hole-nesting species.

Nests from the three warbler species investigated earlier were revisited in order to investigate the variation in hydrological properties of open cup nests. The following hypotheses were tested; 1) Nests with greatest mass and dimensions would have the highest water absorbance and longest drying times 2) nest dimensions influence the drying times and water absorbance properties of warbler nests, and 3) the composition of the materials within nest influence the hydrological properties of warbler nests.

4.2. Materials and Methods

Twelve Blackcap, 21 Reed Warbler, and 23 Willow Warbler nests were used to investigate the hydrological properties of warbler nests. Data for drying times and water absorption of twenty-six nests (10 Blackcaps, 10 Reed Warblers and 8 Willow Warblers) were collected by Lucia Biddle are part of a broader study and are used here with her permission. The data for the hydrological properties of the remaining thirty nests were collected by the author and were used for deconstruction and analysis.

The methodology used here was developed and described by Biddle *et al.* (2018a). Nests were weighed dry prior to soaking using digital scales (Chapter 2). Each nest was placed in a metal sieve resting on a plastic bowl. A bespoke polystyrene lid, sized using cup diameter, was used to cover the cup to imitate an incubating bird. A polystyrene funnel based on each nest's diameter was placed around the nest to trap excess water. This arrangement was to ensure that water falling on the nest was directed away from the cup and through the walls. Each nest was subjected to 245g of water; enough water to be absorbed by large nests which was then fed through a revolving coffee percolator at 30cm height above the nest to ensure the whole nest had equal exposure to water. Nests and all equipment used for the experimental trials were weighed 10 minutes after watering, then at 1 hour, 2 hours, 4 hours, 8 hours and 24 hours after watering. Water absorbed (g) was determined by calculating the difference in original dry nest mass and immediate wet nest mass. Drying time (min) was determined by fitting the total minutes required for the nest to return to original

dry mass to a logarithmic model (based on that reported by McGowan *et al*. 2004).

Nest dimensions (nest mass (mm), nest diameter (mm), nest wall thickness (mm), base thickness (mm)) were collected for all nests as described in Chapter 2. Only a subset of 30 nests were used for deconstruction due to time constraints (see Chapter 2) and were used within the analysis.

All analyses were carried out using Minitab (ver. 17). Data were Log_{10}-transformed prior to analysis. Analysis of variance (ANOVA) was used to determine the effect of species on the mass of water absorbed and the drying time of nests. Post-hoc Tukey's HSD were conducted on statistically significant outputs to identify significant pairwise comparisons if there was a significant effect of species. Spearman's signed-rank correlations were utilised to determine associations between nest dimensions and hydrological properties and to identify associations between nest materials and hydrological properties of nests. Nest materials that were not present in nests of all warbler species here were excluded from statistical analyses. Where significant correlations were detected, analysis of covariance was used to determine the effect of nest dimensions when controlling for species as a factor on nest drying time and the mass of water absorbed. ANCOVA was repeated for nest materials where significant correlations were determined. Where interactions were found to be non-significant they were removed from the model, which was subsequently re-run. The absence of interactions in the results should be taken as indicating a non-significant result.

4.3. Results

The amount of water absorbed by Willow Warbler nests was significantly greater than both Blackcap and Reed Warbler nests ($F_{2,53} = 54.4$, $P < 0.001$, $R^2 = 0.673$; Table 4.1). Reed Warbler nests also absorbed significantly less water than Blackcap nests. Drying times of nests were also significantly ($F_{2,53} = 28.89$, $P < 0.001$, $R^2 = 0.521$) longer for Willow Warbler nests than both Blackcap and Reed Warbler nests, which were not significantly different (Table 4.1). Spearman's signed-rank revealed significant correlations for nest dimensions and hydrological properties of nests. Both water absorbed and drying time showed significant, positive correlations with dry nest mass, nest diameter and

wall thickness (Table 4.2; Fig. 4.1 and 4.2). By contrast, base thickness did not significantly correlate with either of the hydrological measures (Table 4.2).

Dry nest mass was a significant covariate, and species was a significant factor influencing the amount of water absorbed by nests (Fig. 4.1) but not for drying time (Table 4.3). Nest diameter was a significant covariate where species was a significant factor influencing water absorbed by warbler nests (Fig. 4.2) but not drying time (Table 4.5). By contrast, wall thickness was not a significant covariate where species was a significant factor on the two variables (Table 4.5).

Table 4.1. Descriptive statistics (mean±SD) for the hydrological properties of 12 Blackcap, 21 Reed Warbler and 23 Willow Warbler nests. Significantly different values reported by Tukey's HSD output in superscript.

Species	**Hydrological properties**	
	Drying time (min)	Water absorbed (g)
Blackcap	354.5 ± 157.3^{A}	10.26 ± 4.45^{A}
Reed Warbler	477.0 ± 68.0^{A}	7.33 ± 0.534^{B}
Willow Warbler	1024.4 ± 95.2^{B}	19.50 ± 1.35^{C}

Table 4.2. Analysis of Variance (ANOVA) output for species differences in hydrological properties of warbler nests. *F*- values and *P*-values in parentheses.

Factor (df)	**Hydrological properties**	
	Log_{10} drying time (min)	Log_{10} water absorbed (g)
Species (2,53)	28.9 (<0.001)	54.4 (<0.001)
r^2	0.521	0.673

Table 4.3. Spearman's signed rank correlations between nest dimensions and hydrological measures for 56 warbler nests. Spearman's rho values with *P*-values in parentheses.

Nest dimensions	**Hydrological properties**	
	Drying time (min)	Water absorbed (g)
Dry nest mass (g)	0.649 (<0.001)	0.650 (<0.001)
Nest diameter (mm)	0.554 (<0.001)	0.807 (<0.001)
Wall thickness (mm)	0.596 (<0.001)	0.731 (<0.001)
Base thickness (mm)	0.232 (0.085)	0.068 (0.618)

Table 4.4. Spearman's signed rank correlations between nest materials and hydrological measures for 30 Warbler nests. Spearman's rho values with P-values in parentheses. Shaded cells indicate significant correlations.

Nest materials (g)	**Hydrological properties**	
	Drying time (min)	Water absorbed (g)
Grass	0.265 (0.158)	-0.131 (0.492)
Moss	0.547 (0.002)	0.577 (0.001)
Hair	0.358 (0.052)	0.146 (0.441)
Feather	0.697 (<0.001)	0.623 (<0.001)
Leaves	0.082 (0.668)	0.080 (0.676)
Roots	0.170 (0.368)	0.403 (0.027)

Table 4.5. ANCOVA for the effect of species as a factor with nest dimensions as a covariate on the hydrological measures on 56 Warbler nests. *F*-values with *P*-values in parentheses.

Hydrological properties	Species (2,52)	Log_{10} Nest mass (1,52)	r^2
Log_{10} drying time (min)	7.55 (<0.001)	1.37 (0.248)	0.534
Log_{10} water absorbed (g)	21.4 (<0.001)	4.25 (0.044)	0.697
	Species (2,52)	Log_{10} Wall thickness (1,52)	r^2
Log_{10} drying time (min)	10.4 (<0.001)	3.43 (0.070)	0.551
Log_{10} water absorbed (g)	9.68 (<0.001)	3.56 (0.065)	0.694
	Species (2,52)	Log_{10} Nest diameter (1,52)	r^2
Log_{10} drying time (min)	14.3 (<0.001)	2.01 (0.162)	0.539
Log_{10} water absorbed (g)	7.93 (0.001)	10.8 (0.002)	0.799

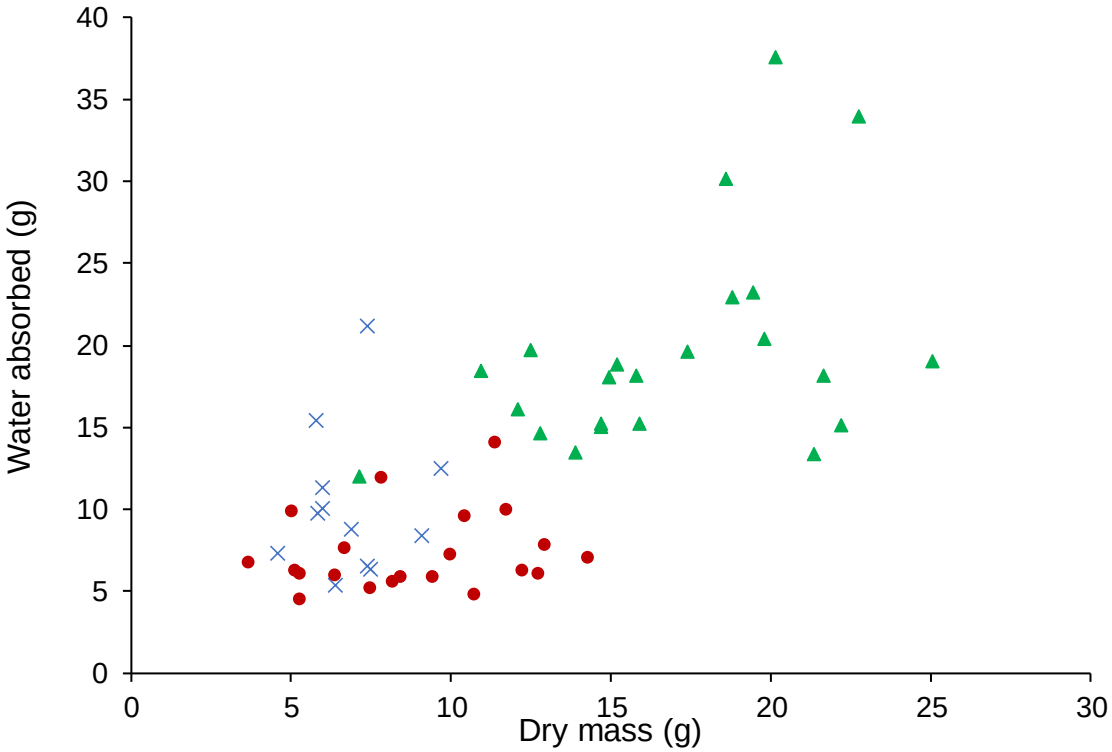

Figure 4.1. Relationship between dry nest mass (g) and the mass of water absorbed (g) by 56 Warbler nests. Blue squares= Blackcap, Red circles= Reed Warbler, Green triangles= Willow Warbler.

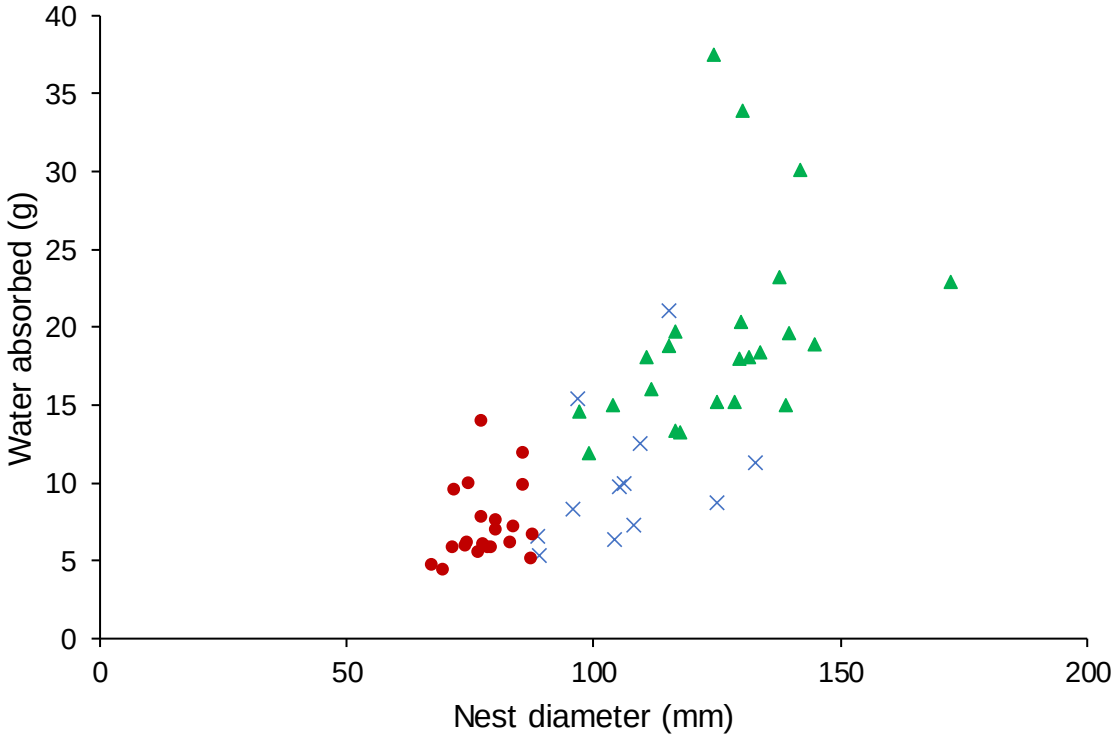

Figure 4.2. The effect of nest diameter (mm) on the mass of water absorbed (g) by 56 Warbler nests. Blue squares= Blackcap, Red circles= Reed Warbler, Green triangles= Willow Warbler.

Table 4.6. ANCOVA for the effect of species as a factor with nest materials as a covariate on the hydrological measures on 30 Warbler nests. F-values with P-values in parentheses.

	Covariate	Species	Material	R^2
Log_{10} drying time (min)	DF	(2,26)	(1,26)	
	Log_{10} (Moss)	1.59 (0.223)	1.09 (0.306)	0.549
	Log_{10} (Feather)	8.50 (0.001)	0.34 (0.562)	0.537
Log_{10} water absorbed (g)				
	Log10 (Moss)	0.76 (0.476)	2.55 (0.122)	0.585
	Log10 (Feather)	9.85 (0.001)	0.90 (0.350)	0.560
	Log10 (Roots)	14.3 (<0.001)	2.89 (0.101)	0.590

For the subset of nests that were deconstructed Spearman's signed-rank revealed significant correlations between nest materials and hydrological measures (Table 4.4). Moss and feather mass were significantly, positively correlated with both drying time and water absorbed by warbler nests (Table 4.4). Mass of roots also showed a significant positive correlation with water absorbed (Table 4.4). Mass of grass, hair, leaves and artificial materials were not significantly correlated with any hydrological properties (Table 4.4).

Moss (g) was not a significant covariate, nor was species a significant factor influencing either water absorbed or drying time by warbler nests (Table 4.5). Similarly feather mass was not a significant covariate on hydrological properties of Warbler nests however species was a significant factor. Mass of roots was not a significant covariate on water absorbed where species was a significant factor (Table 4.5).

4.4. Discussion

Intraspecific variation in the hydrological properties of nests exists between warbler species. The mass of water that nests were capable of absorbing was significantly different between Willow Warblers, Reed Warblers and Blackcaps; Willow Warblers absorped a significantly greater mass of water than Reed Warbler and Blackcap nests. Moreover, interspecific variation was exhibited in the drying time of warbler nests; Willow Warbler nests had significantly slower drying times that Reed Warbler and Blackcap nests. Hydrological properties of warbler nests were significantly correlated with nest dimensions and specific materials used within the nest.

Although species affected how much water was absorbed, and drying times, this may simply reflect nest mass, as heavier nests may absorb more water (Rohwer and Law 2010). Building a nest with a large diameter and mass may prevent the absorbed water from reaching the nest cup. Building a nest with a small overall diameter may also be beneficial in the case of Reed Warblers where a large nest could be difficult to suspend. Under the assumption that the incubating bird covers the cup during periods of rainfall, a nest with a small diameter and thin walls could absorb less water overall, preventing water entering the nest cup. Furthermore, Willow Warbler nests needed almost double the time to dry than Blackcap and Reed Warbler nests which may have been influenced by the Willow Warbler nest's large diameter reducing the rate of water loss.

Specific nest materials did not significantly influence the drying time of nests, which was also found by Slagsvold (1989b) for nest drying times. However unmeasured factors, such as interspecific variation in the structural properties of the nest wall, may have affected nest drying time. Greater water absorption did not necessarily result in longer nest drying time; Blackcap nests produced the shortest drying times even though Reed Warbler nests absorbed less water mass than Blackcap nests. Reed Warbler nests were composed of tightly woven dry grass, creating a dense nest wall when compared to Blackcap nests which have a multitude of small gaps within the walls (Chapter 2, Fig 2.1). Slagsvold (1989b) suggested that loosely constructed nests dried out faster than denser nests. More water may be trapped in Blackcap nest walls due to the large nest diameter however the small gaps may allow for the water to evaporate faster than that of trapped water in Reed Warbler nests. Gaps may also provide a drainage method

to prevent rain water entering the cup and could minimise the exposure of the offspring to water after rainfall. Interspecific variation in nest wall structure has been considered in terms of thermoregulatory function of nests and is still yet to be tested in regards to the hydrological function of nests (Heenan and Seymour 2012, Deeming and Biddle 2015). Kern (1984) found a significant effect of nest wall porosity on thermal conductance and interspecific variation in different subspecies of White-Crowned Sparrow *Zonotrichia leucophrys* nests. Direct measurement of interspecific variation in nest wall structure and porosity may allow for a wider understanding of nest construction in relation to hydrological properties. Nest materials have been shown to influence other nest functions such as the thermal properties of nests but there is also interspecific variation in the extent of the effect of certain nest materials on nest function (Hilton *et al.* 2004; McGowan *et al.* 2004; Deeming and Mainwaring 2015). Although nest materials do not significantly influence the hydrological properties of Warbler nests, further investigation of the effect of materials on nest hydrological properties in other passerine species may develop a clearer understanding of the use of certain materials in passerine nests. A larger sample size than used in this study for each species may also reveal effects of nest materials on the hydrological properties of nests.

The effect of species on hydrological properties of Warbler nests may reflect species differences in nest site and constructing a nest to minimise adverse effects of water on the nest microclimate at the nest site. Willow Warblers are ground nesting species, whereas Reed Warblers suspend their nests above the ground and Blackcap nests are usually found in low trees and bushes (Tiainen *et al*. 1983; Glutz von Blotzheim and Bauer 1991; Cramp 1992; Morrison *et al.* 2015). Although Reed Warbler nests are suspended above the ground so are not at threat from water pooling, they may be built to prevent heavy rainfall entering the nest to ensure the reed stems are still capable of holding the weight of the nest. Blackcap nests may be protected from rainfall by nesting in bushes and from the tree canopy above. Although many ground nests are under vegetative cover, ground nests are likely to be exposed to water pooling around the nest as well as experiencing precipitation from above which could alter the nest microclimate (Morrison *et al.* 2015). Passerine nests that are constructed above the ground are unlikely to be exposed to water from below, with the only

exposure being from rainfall. It would perhaps be beneficial for the nest to be constructed to prevent water entering from above pooling inside the nest walls and seeping into the cup. The majority of the Willow Warbler nest wall is constructed with moss. Although moss was not a significant covariate, there was a positive correlation between the mass of moss and water absorbed. Wesolowski (1996) suggested that moss used to construct "bulky foundations" of cavity nests allows for absorption of tree sap, which may also be used by ground nesting species to prevent pooled water entering the cup from the ground (Morrison *et al.* 2015). Building a nest that is capable of absorbing large amounts of water may seem detrimental to the maintenance of optimal nest microclimate, however it is perhaps the most effective way for ground nesting species to cope with high precipitation levels.

Investigating the hydrological properties of the nests of a wider variety of passerine species would be beneficial for developing a clearer understanding of the relationship between nest construction and function, and to test interspecific and intraspecific variation in nest function. Slagsvold (1989b) investigated the interspecific variation in water retention capability of six passerine nests. Rohwer and Law (2010) found that intraspecific variation in nest hydrological properties exists for Yellow Warbler nests from different geographical locations. Biddle *et al.* (2018a) also found interspecific variation in nest hydrological properties between species from the Fringillidae and Turdidae families.

Developing a standardised method to measure the hydrological properties of nests would be advantageous as it would allow for better cross examination of studies. The method of submerging nests in a laboratory and calculating drying times has been utilised by previous studies (Slagsvold 1989b, Rohwer and Law 2010). Submerging nests allowed for insight into the differences in water absorption between nests and indicated whether certain nest materials allow water to be absorbed (Slagsvold 1989b). However, *in situ*, nests are unlikely to be exposed to the level of soaking exhibited by submerging nests in the laboratory. Slagsvold (1989b) submerged nests of species that nest above ground which may not be a biologically relevant method as they are unlikely to be flooded by water pooling from below. Nests may not be designed to absorb the amount of water during soaking over a brief period and drying times may not be representative of a nest's drying time in the natural world. This study

attempted to mimic the extent of rainfall a nest would experience in the natural world whilst still measuring water absorption and nest drying times. The advantage of mimicking a light rain shower rather than submerging nests is that certain nest materials could exhibit their function as they may do *in situ*. Conducting hydrological trials in a laboratory is useful when controlling for humidity and temperature changes that could influence data for drying times. Studies have tested the effect of rainfall on nestling development *in situ* but have not directly investigated the hydrological function of nests *in situ*. A long-term *in situ* investigation of precipitation levels, nest construction and nestling development may be useful to develop a clearer understanding of the responses of birds to precipitation levels in terms of nest construction and whether nestling development is affected by rainfall.

In conclusion, the hydrological properties of warbler nests show interspecific variation in both drying times and the mass of water absorbed which is likely a reflection of the nest site exhibited by each species. Investigating unmeasured factors here such as nest wall porosity would be a fruitful area of research that could provide an insight into how nests are constructed to mitigate the adverse effects of moisture and rainfall. *In situ* studies would also provide an insight into the plasticity and adaptive behaviours exhibited by birds due to changes in environmental conditions. There remains a lack of understanding in regards to the effect of rain on the breeding biology of birds. Extreme changes in weather and precipitation levels is a major concern for ecologists and conservationists thus it is unclear as to the complexity of the effect of climate change on avian biology. Studying nests from various continents may also be beneficial as the effect of rainfall on avian breeding biology may vary across continents due to potential variation in precipitation levels and seasonal phenomena (Arnell 1998; Cai *et al.* 2014). This study adds to the growing field of research around nest construction and function but investigating more species is still required to develop a clearer understanding of nest functionality.

5.1. Functional variability in warbler nests

Nest morphology has been documented for multiple species of birds but very few species have been quantitatively investigated in terms of their dimensions and the materials used to construct the nests (Elts 2005; Britt and Deeming 2011; Deeming and Mainwaring 2015; Briggs and Deeming 2016; Taberner Cerezo and Deeming 2016; Biddle *et al*. 2017, 2018a). This thesis contributes new quantitative data for Blackcap, Reed Warbler and Willow Warbler nest morphology.

Blackcap nests were the lightest in mass of the species studied and were mainly constructed with dry grass supplemented by other types of plant material. The cup lining was also dominated by grass species but around 20% of the cup lining mass was compromised by animal hair and plant roots. These Blackcap nests had walls that were capable of reducing the effect of air movement on the internal cup microclimate despite being loosely constructed with many visible gaps in the nest structure. Trapped air within the nest walls of other passerine species nests can provide a layer of insulation (Deeming and Biddle 2015). Therefore, the gaps within Blackcap nests structure may allow for an insulating layer of trapped air (Deeming and Biddle 2015). Following exposure to water, Blackcap nests dried out the fastest of the three warbler species and absorbed half the mass of water than that of Willow Warbler nests. The many holes in the Blackcap nest structure may allow rain water to drain through the walls rather than being absorbed intones nest cup. Loosely constructed walls in Blackcap nests may also contribute to the fast drying times which could be beneficial in maintaining an optimal nest microclimate. Blackcap nests may also be protected from precipitation and wind by the shrubs and tree canopies at the nest site so may not require a large nest or great amount of insulating nest material to provide protection.

Willow Warbler nests were the heaviest nests and were constructed predominantly with dry grass and moss but also animal derived materials such as hair and feathers. The cup lining was mostly grass although 30% of the cup lining was composed of animal derived materials and moss. Although constructed differently Willow Warbler nests were in some ways similar to

Blackcap nests in that they also reduced the effect of air movement on the internal cup microclimate; however, it is unclear whether this was due to the thermal inertia of the nest walls or trapped air within the nest. Although Hilton *et al*. (2004) found grass to have a relatively low insulatory value in Common Blackbird *Turdus merula* nests it was associated with better insulation observed in more northerly nest cups (Mainwaring *et al.* 2014b). Willow Warbler nests had the highest water absorbance and longest drying times of the three species described here, which may reflect the large nest size.

Reed Warbler nests were mostly constructed of dry grass, such as reed, which may reflect the abundance of this material in their habitat (Briggs and Deeming 2016). It also can be easily manipulated around reed stems to allow for suspension. Reed Warbler nest walls were capable of reducing the effect of air movement on the internal microclimate of the nest and were associated with the lowest cooling rates of all three species studied. The nest walls did not have visible gaps and this high density may contribute to the reduced cooling rates observed in Reed Warbler nests. Reed Warbler nests absorbed the least water of the three species here, which may be due to the small nest diameter. Reed Warbler nests are suspended on stems in reed beds, therefore may be exposed to some level of precipitation and wind but are protected from the ground pooling of water so would not require a large base thickness or a large nest diameter to absorb the water.

Nest morphology has been previously reported for three *Phylloscopus* warbler species – Wood Warbler, Chiffchaff and Willow Warbler – from different geographical regions (Tiainen *et al.* 1983). Unlike Willow Warbler and Chiffchaff nests, Wood Warblers did not incorporate feathers into the nest lining and had the poorest insulation of the three species, which probably reflected its southerly distribution as more southerly nests are likely to be exposed to higher ambient temperatures thus may require less insulating nest materials (Tiainen *et al*. 1983). By contrast, it is reported here that feather mass was not a significant factor in contributing to nest insulation in the warbler nests studied. Future studies could investigate whether any characteristics in nest morphology are specific to the *Acrocephalus*, *Sylvia* or *Phylloscopus* genera (or any other genera within the Sylvidae). Comparison of nest materials used to build warbler nests from various geographical locations that experience different precipitation and rainfall levels

would provide more insight into the adaptability and plasticity of nest building behaviours of passerines in different natural environments (Kern 1984; Britt and Deeming 2011; Deeming *et al*. 2012; Mainwaring *et al*. 2012; Biddle *et al*. 2018).

5.2. Factors affecting intra- and interspecific variation in the composition of passerine nests

Intraspecific variation exists in the quantity of nest materials incorporated into nests of other passerine species (Kern 1984; Britt and Deeming 2011; Surgey *et al.* 2012; Mainwaring *et al.* 2014; Biddle *et al*. 2015; Deeming and Mainwaring 2015; Taberner Cerezo and Deeming 2016; Biddle *et al.* 2018). Blue Tits have been shown to vary the mass of material within the cup lining due to temperature changes (Mainwaring and Hartley 2008). Common Blackbird nests have also been shown to vary in the mass of grass used within the cup lining (Mainwaring *et al*. 2014).

Interspecific variation also exists in the variation and amount of materials used to construct nests, for example Common Blackbirds construct nests with only plant derived materials (Mainwaring *et al.* 2014; *Biddle et al.* 2015) whereas some species such as Blue Tits, Great Tits and Long Tailed Tits incorporate various animal derived materials within their nests which may also vary in mass within the nest (McGowan *et al.* 2004; Mainwaring and Hartley 2008; Britt and Deeming 2011; Mainwaring *et al.* 2012).

Although this thesis contributes to the growing knowledge base of quantified nest materials, quantification of nest materials remains incomplete or unavailable for the majority of passerine species. Further quantification of nest materials for many more nests is encouraged as it may allow insight into the scope of intraspecific and interspecific variation in passerine nest construction. Moreover, investigating the relationship between nest morphology and the functional properties of nests may be aided by quantification of nest materials as it allows for a true representation of the construction of the nest, whether birds are building their nests in a selective manner, due to environmental conditions or due to the availability of materials in the environment. Further studies attempting to quantify nest materials could experimentally add nest materials for the sample of birds to use to investigate whether nest materials are chosen selectively or due to availability.

In situ observations of nest building behaviours are scarce for many species, which is likely due to the difficulty in collecting data from wild bird populations but may be useful to identify various construction methods that species use, and the data may be used for intra- and interspecific comparisons (Healy *et al.* 2015). Several nest construction methods by birds have been reported (Hansell 2000). For example, birds may use saliva to stick nest components together which aids the moulding of the mud cup in House Martin *Delichon urbicum* nests (Silva *et al*. 2010). Birds may also pile materials up to build a nest, for example, White-tipped Brown Jay *Psilorhinus mexicanus* nests are constructed by building a platform of layered twigs (Skutch 1960). Weaverbirds such as Village Weavers *Ploceus cucullatus* weave grasses together to form their nests (Collias and Collias 1964; Walsh *et al*. 2010, 2011). Observing the construction methods of warbler nests could allow further insight into the interspecific variation in nest regions of closely related species. Overcoming some of the difficulties in observing nest building behaviours in wild populations should be a focus for researchers attempting to quantify nest building behaviours. Identifying and locating breeding pairs in a wild population may prove difficult, and observing nest building behaviours across the breeding season could be a lengthly process.Video recording and tracking breeding pairs may enable researchers to develop methodologies to observe nest building behaviours.

It has been shown previously that nest regions can be identified by materials used (Britt and Deeming 2011; Biddle *et al*. 2015, 2017; Briggs and Deeming 2016; Taberner Cerezo and Deeming 2016). In species that incorporate animal derived materials within their nests, such as Great Tits and Blue Tits, animal derived materials are found in a greater proportion within the cup than the outer nest where there is a greater proportion of plant material (Mainwaring and Hartley 2008; Britt and Deeming 2011). In species that use plant derived materials only, such as Common Blackbirds, the outer nest materials are stronger, thicker and more rigid than those in the cup, suggesting that Blackbirds are selective in the materials they use for each nest region (Biddle *et al*. 2015). The outer nest materials of Bullfinch nests are also stronger and coarser than those within the cup (Biddle *et al.* 2017). Captive Zebra Finches *Taeniopygia guttata* have been shown to select for materials of certain physical characteristics

such as colour (Muth and Healy 2013) and structural properties (Bailey *et al*. 2014). Reed Warbler nest regions were not identifiable by the type of materials used but may be distinguishable by other unmeasured factors such as the method of construction, such as weaving or moulding, used to build the cup lining and outer nest regions. Reed Warbler nests may not have a distinguishable outer nest and cup lining in an effort to reduce conspicuousness of the nest to predators and parasitic species such as Cuckoos *Cuculus canorus* (Moksnes *et al.* 2000).

An interesting area to explore may be the use of various construction methods within the nest and whether these contribute to the functional properties of nests. Structural properties in various regions within nests such as nest porosity and density may be due to certain construction methods, which could perhaps contribute to the thermal and hydrological properties of nests. Furthermore, interspecific variation in nest construction methods (Kern 1984; Crossman *et al*. 2011; Heenan and Seymour 2012) could contribute to variation in nest wall structure and porosity, and perhaps functional properties of nests.

Further investigation into the opportunistic and selective use of nest materials may also provide evidence for the adaptability of nest building, which may also benefit from *in situ* observations. Briggs and Deeming (2016) suggested that Pied Flycatchers were selective in the use of moss within their nests but were opportunistic in the species of leaves that were incorporated into their nests. Surgey *et al.* (2012) also suggested that Great Tits were opportunistic in the use of artificial materials but were also selective in that they preferred wool-like artificial materials.

In situ observations in other species may provide a clearer insight into the distances and locations birds are prepared to travel to in order to collect their nest materials, which may also contribute further evidence for the energetics of nest building (Surgey *et al.* 2012; Nord and Williams 2015).

The use of artificial materials in nests has been reported for a few species, but it is unclear as to whether they have been selected for certain functions or they are mistaken for similar, naturally occurring materials (Sergio *et al*. 2011; Surgey *et al*. 2012; Suarez-Rodriquez *et al.* 2013). The incorporation of cigarette butts within House Finch *Carpodacus mexicanus* and House Sparrow *Passer domesticus* nests was suggested to be selective because of their anti-parasitic properties (Suárez-Rodríguez *et al*. 2013). The artificial materials found in

Blackcap and Reed Warbler nests here were typically sections of string such as baling twine. String or baling twine may be mistaken for long grass or roots and may be abundant near sources of dry grass such as hay bales. Whether the use of artificial materials in Reed Warbler and Blackcap nests was opportunistic or selective for their characteristics requires further investigation. Experiments on nest material preference of captive Zebra Finches have been conducted using artificial materials such as string (Bailey *et al*. 2014) but it would also be interesting to investigate whether Zebra Finches can distinguish between natural and artificial materials. Whether wild birds also have a preference or select for either natural or artificial materials could also be a useful in developing a clearer insight into the use of artificial materials in avian nests.

5.3. Functional properties of nests

5.3.1. Thermal properties

All three species of warblers built nests that reduced the effect of air movement on the internal nest microenvironment. Blackcap nests were the least insulating nest of the three species tested but the walls still acted as an effective buffer between the internal cup microclimate and the external environment, even during periods of air movement. Gray and Deeming (2017) also found a similar effect of air movement on nests from the Fringillidae and Motacillidae. Nest insulatory values increased for all nests, as was the case here, but the nests did not gain insulation in absolute terms as the cooling rates inside nests increased also (Gray and Deeming 2017). Air movement did have an effect on the internal microenvironment of nests from all species during periods of air movement. Here, warbler nests with a larger mass were better at minimising the effect of air movement on the internal microclimate of the nest. Similarly, Gray and Deeming (2017) found a significant effect of nest mass on the difference in cooling rate between still air and moving air. Although Gray and Deeming (2017) investigated the effect of nest dimensions on thermal properties during periods of air movement but did not test the effect of nest materials. Rather than relying on observations of what birds do, future studies could use captive species to experimentally test the effect of different nest materials on the thermal properties of nests constructed. Studying captive birds can be advantageous over wild

populations of birds as conditions can be well controlled and experiments can be easily manipulated. Researchers should be cautious when comparing studies of captive birds as the observed behaviour smay not be entirely representative of the wild population.

The thermal properties of nests were also different for the three warbler species, which may arise from unmeasured factors such as interspecific variation in nest wall structure. Investigating the structure of the nest wall in terms of porosity and density may allow a clearer insight into how nests are constructed to mitigate the adverse effects of environmental conditions on the developing offspring. Previous studies have suggested that nest porosity, density, thickness and air gaps influence the thermal properties of nests (Kern 1984; Rohwer and Law 2010; Crossman *et al*. 2011; Heenan and Seymour 2012; Deeming and Biddle 2015). Nest porosity has previously been measured by light penetration (Skowron and Kern 1980; Kern 1984) which could be applied to measuring the porosity of warbler nests in future studies. Determining interspecific variation in nest wall porosity and density of warbler nests may provide an insight into the construction of warbler nests in relation to nest insulation.

Nest building and incubation are costly behaviours for parent birds (Nord and Williams 2015). Interspecific variation in parental care may have an influence on the nest thermal properties. Building a small nest may allow for energy conservation during construction and a largely insulating nest may not be required if the parents are able to share energy expenditure between each other. By contrast, in species that exhibit uniparental care the nest may need to be highly insulating to compensate for the energetic demands of the sole incubating and chick rearing parent (Nord and Williams 2015; Deeming 2016). Slagsvold (1989b) suggested that a smaller nest cup is beneficial for successful hatching, but a large nest is advantageous for the survival of chicks during rearing periods. Nest size may also be influenced by predator avoidance, for example, predation pressures were more intense for larger Common Blackbird nests in comparison to smaller nests, larger nests are less advantageous (Wysocki *et al*. 2015). Building a smaller nest may therefore be beneficial in both predator avoidance and energy conservation during construction, which may allow for greater energy expenditure during incubation and may improve reproductive success in some species. Further investigation of the relationship between the energetics of

parental care and nest thermal properties, including the construction of the nest for thermoregulatory function, may be useful in developing a clearer insight into how the costs of incubation and chick rearing influence nest function.

Developing a standardised method to investigate the thermoregulatory function of passerine nests would be advantageous when comparing studies and species. Methods of investigating the thermal properties of nests have improved over time and procedures have changed accordingly to the available research. Previously, several studies that investigated nest thermal properties using temperature loggers pushed the loggers into the nest material (Mainwaring *et al.* 2012; Mainwaring *et al.* 2014b; Deeming and Biddle 2015; Taberner Cerezo and Deeming 2016). Deeming and Gray (2016b) were the first reported study to invert nests and temperature loggers rested on the internal surface of the cup lining rather than being pushed inside the nest lining material, the method that was adopted here. The two methods may produce different results as pushing the temperature logger into the material may produce slower cooling rates than when the material is resting on the cup thus care should be taken when cross-examining reported thermal measures. Moreover, pushing the temperature loggers into the nest material may not be as biologically relevant than allowing the temperature loggers to rest on the internal cup surface, as the clutch would typically rest on the cup lining surface. The influence of different nest structures may also vary between both studies, for example floor thickness may have a greater influence on the cooling rate of the temperature logger if it is pushed in the nest material rather than resting on the surface. Previous studies have used metal conductors such as heat flux transducers and dummy eggs which may be more biologically relevant than a flat temperature button. The direction of heat energy transfer from a dummy egg may be more representative of that of an avian egg than the flat temperature button (Skowron and Kern 1980; Ar and Sidis 2002; Heenan and Seymour 2012). The direction of heat energy transfer through the nest walls and floor may also be more representative from a sphere than a flat temperature logger.

Insulatory values are useful for interpreting relative differences in insulation between test conditions however the values are also contextual. As discussed by Gray and Deeming (2017) insulatory values represent the relative difference between the cooling rate of temperature loggers inside the nest and

outside the nest, thus are useful for confirming whether nests act as buffers again changing conditions. However, there should be caution taken when comparing insulatory values reported for different studies to ensure that the results are comparable. Testing location is also an important factor to consider when carrying out thermal trials (Chapter 3). Performing trials in an enclosed, controlled test chamber, such as the wind tunnel used in Chapter 3, may allow for improved accuracy of results due to the variability of conditions that may arise in an open room such as a laboratory. Hence, future studies should consider conducting thermal trials in an air tight location or an enclosed chamber.

5.3.2. Hydrological properties

Nest morphology has been suggested to be an important factor in allowing nests to cope with water. Previous studies suggested that passerine nests with larger walls and a greater nest mass absorbed more water and took longer to dry (Slagsvold 1989b; Rohwer and Law 2010). By contrast other studies have suggested that large nest walls and bases with a high proportion of moss may aid water absorption (Wesolowoski 1996; Wesolowski *et al.* 2002). The presence of moss within some passerine nests had a significant influence on nest drying times but the effect of this on the nest microclimate is unclear (Biddle *et al.* 2018). For warbler nests, moss was not a significant factor influencing nest hydrological properties although there was a significant correlation between moss and nest drying times and also the mass of water absorbed by nests. The influence of nest materials, such as moss, on the hydrological properties of nests may not be apparent here as only a small sample of each species was deconstructed for quantification of their composition. Silk was the only nest material to influence the water absorbed by nests, but was a rare material found on the outer nest of Reed Warbler and Blackcap nests. This may be more indicative of the effect of the outer nest structure on the hydrological properties of nests.

Investigating the influence of nest wall density and porosity on the hydrological properties of nests found above ground may aid in interpreting interspecific variation in water absorption and nest drying time. That nests of the three species investigated here had different hydrological properties may also be indicative of the interspecific difference in their nest site. For example, species such as Blackcaps that build nests that are above ground perhaps build nests

with a loose structure creating gaps to act as a drainage system. By contrast, Willow Warblers are ground nesting species which have the threat of precipitation above and from pooling underneath so perhaps build the nest as a large sponge to prevent water entering the cup. A future direction of research would be to compare the hydrological properties of nests from multiple species that nest above and on the ground and to investigate the effect of nest site on the interspecific differences in nest wall structure. Nests from species that vary in nest site, such as European Robin nests, may also provide evidence for intraspecific variation in nest hydrological properties at different nest heights. The effect of water on the thermal properties of nests would also be useful in understanding how the internal microclimate may be affected by precipitation and moisture. Investigating a relationship between the presence of materials such as moss within a nest and the internal microclimate, and potentially nestling survival may be useful in understanding the effects of nest drying time on reproductive fitness.

Studies that have previously investigated the hydrological properties of nests by submerging them in water and allowing them to dry (Slagsvold 1989b; Rowher and Law 2010) and may not be a biologically relevant method as nests are unlikely to experience that amount of soaking over a short period of time. Nests that are built above ground are also unlikely to be flooded so submerging nests may not give a true representation of the degree of water absorbance or subsequent drying times as would be observed *in situ*. Further studies that investigate the hydrological properties of nests should consider using methodologies that simulate the volume of precipitation a nest would experience in its natural environment. A long term *in situ* study could investigate variation in the construction of nests in response to changing precipitation and moisture levels. Such data could allow for a clearer insight into how birds adapt nest building behaviours to minimise the effect of environmental conditions on offspring survival.

5.4. Conclusions

In conclusion, closely related species of passerines show interspecific variation in nest construction and the functional properties of nests. This thesis has presented new empirical evidence for the interspecific variation in nest morphology of passerine nests. Newly quantified data for the composition of nests for three warbler species adds to the growing knowledge base of quantitative data for the materials used within avian nests. The outer nest and nest cup lining may be characterised by the type and amount of materials used within the regions for some species, supporting previous suggestions that specific materials have certain functional properties in avian nests. Air movement can affect the internal microclimate of warbler nests but nests are able to minimise the effect of air movement. This thesis provides support for the relationship between nest mass and the thermal properties of passerine nests. Animal derived materials present within warbler nests did not significantly contribute to nest insulation, contradicting previous findings for some other passerine species. Interspecific variation is exhibited in the hydrological properties of warbler nests, which may be reflective of the variation in nest sites between closely related species. Nests from closely related warbler species can be diverse in the location of nests, the type of habitat and nest environment (Cramp 1992; Leniowski and Węgrzyn 2014; Morrison *et al*. 2015). Moreover, closely related warbler species build nests which exhibit strong interspecific variation in nest morphology but can still reduce the effect of air movement on the internal microclimate of the nest. By contrast, warbler species can be diverse in their hydrological properties which may be due to their nest location. There remain gaps within the knowledge of how birds construct their nests in order to perform functions that may be advantageous to reproductive success. Therefore, further research is still needed to develop a clearer understanding of the role of avian nests in relation to parental and offspring survival. It is recommended that research should be directed towards how birds respond to the environmental conditions at the nest site to overcome environmental pressures to ensure reproductive success.

Acknowledgements

Firstly, I would like to thank Dr. Charles Deeming and Dr. Adrian Goodman for their excellent supervisory support, from departure of knowledge to invaluable feedback. It has been a privilege to study under the academic supervision of Dr. Charles Deeming throughout both my Undergraduate and Post-graduate courses.

I am grateful to Lucia E. Biddle for her guidance during data collection and transfer of knowledge in the functional properties of nests. I would also like to thank Liberty. A. Gray for her guidance, especially in wind tunnel methodologies.

This thesis was also made possible thanks to the members of the British Trust for Ornithology Nest Record Scheme that spent their time observing and collecting warbler nests used in this thesis. Many thanks to Nick Davies and Charles Deeming for collection of Reed Warbler nests from Wicken Fen.

I wish to express my sincerest thanks to my family, especially my parents, Linda and David Dickinson, for their kindness, love and support. I extend my gratitude to David Brockton for his support and encouragement over the past 24 months.

I am indebted to my colleagues at St Katherine's Care for their generous flexibility and support. To my colleagues at Forest Assets, thank you for the use of a quiet office space and a continuous supply of coffee.

Finally, I am privileged to acknowledge the memory of John. P. Davies. His dedication to hard-work, interest and respect for the animal kingdom continues to inspire and resonate during my time at university.

References

Akresh, M.E., Ardia, D.R. and King, D.I. (2017). Effect of nest characteristics on thermal properties, clutch size, and reproductive performance for an open-cup nesting songbird. *Avian Biology Research*, **10**, 107-18.

Álvarez, E., Belda, E.J, Verdejo, J. and Barba, E. (2013). Variation in Great Tit nest mass and composition and its breeding consequences: a comparative study in four Mediterranean habitats. *Avian Biology Research*, **6**, 39-46.

Ar, A. and Sidis, Y. (2002). Nest microclimate during incubation. In DC Deeming, ed. *Avian incubation: behaviour, environment, and evolution*, pp. 143-60. Oxford University Press, Oxford.

Ar, A. and Deeming. D.C. (2009). Roles of water and gas exchange in determining hatchability success. *Avian Biology Research*, **2**, 61-6.

Arnell, N. W. (1998). The effect of climate change on hydrological regimes in Europe: a continental perspective. *Global Environmental Change,* **9**, 5-23.

Bailey, I.E., Morgan, K.V., Bertin, M., Meddle, S.L. and Healy, S.D. (2014). Physical cognition: birds learn the structural efficacy of nest material. *Proceedings of the Royal Society B*, **281**, e20133225.

Biddle, L.E., Deeming, D.C. and Goodman, A.M. (2015). Morphology and biomechanics of the nests of the Common Blackbird *Turdus merula*. *Bird Study*, **65**, 87-95.

Biddle, L. E., Goodman, A. M. and Deeming, D. C. (2016). Geographical effects on the mass and dimensions of finch (Fringillidae, Passeriformes) and thrush (Turdidae, Passeriformes) nests. *Avian Biology Research*, **9**, 13-21.

Biddle, L.E., Goodman, A. M. and Deeming, D. C. (2017). Construction patterns of birds' nests provide insight into nest-building behaviours. *PeerJ*, **5**, e3010.

Biddle, L.E., Deeming, D. C. and Goodman, A. M. (2018). Functional properties of nests of the Turdidae and the Fringillidae. Unpublished.

Blackman, R., Deeming, D.C., Eady, P.E. and Barton, S. (2006). Egg cooling in nests and attentiveness during incubation in British Passerines. *Avian and Poultry Biology Reviews*, **17**, 57-76.

Boulton, R.L. and Cassey, P. (2012). How avian incubation behaviour influences egg surface temperatures: relationships with egg position, development and clutch size. *Journal of Avian Biology*, **43**, 289-96.

Briggs, K.B. and Deeming, D.C. (2016). Use of nest materials in construction by Pied Flycatchers *Ficedula hypoleuca* reflects localized habitat and geographical location. *Bird Study*, 1-9.

Britt, J. & Deeming, D.C. (2011). First-egg date and air temperature affect nest construction in Blue Tits *Cyanistes caeruleus* but not in Great Tits *Parus major*. *Bird Study,* **58**, 78-89.

Cai, W., Borlace, S., Lengaigne, M., van Rensch, P., Collins, M., Vecchi, G., Timmermann, A., Santoso, A., McPhaden, M. J., Wu, L., England, M. H., Wang, G., Guilyardi, E. & Jin, F. (2014). Increasing frequency of extreme El Nino events due to greenhouse warming. *Nature Climate Change*, **4**, 111-116.

Collias, E. C. & Collias, N. E. (1964). The development of nest-building behaviour in a weaverbird. *The Auk*, **81**, 42-52.

Collias, N. E. and Collias, E.C. (1984). *Nest building and bird behaviour*. Princeton University Press, Princeton.

Cramp, S. (1992). *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic*. Vol. VI. Warblers. Oxford University Press, Oxford.

Crook, J.H. (1963). A comparative analysis of nest structure in the weaver birds (Ploceinae). *Ibis*, **105**, 238-62.

Crossman, C.A., Rohwer, V.G. and Martin, P.R. (2011). Variation in the structure of bird nests between northern Manitoba and southeastern Ontario. *PLoS ONE*, **6**, e19086.

Dawson, R.D., O'Brien, E.L. and Mlynowski, T.J. (2011). The price of insulation: costs and benefits of feather delivery to nests for male tree swallows *Tachycineta bicolor*. *Journal of Avian Biology*, **42**, 93-102.

Deeming, D.C. (2002a). *Avian incubation: behaviour, environment, and evolution*. Oxford University Press, Oxford.

Deeming, D.C. (2013). Effects of female body size and phylogeny on avian nest dimension. *Avian Biology Research*, **6**, 1-11.

Deeming, D.C. (2016). How does the nest-incubation unit work? *Avian Biology Research*, **9**, 103-13.

Deeming, D.C., Mainwaring, M.C, Hartley, I.R. and Reynolds, S.J. (2012). Local temperature and not latitude determines the design of Blue tit and Great tit nests. *Avian Biology Research*, **12**, 203–8.

Deeming, D.C. and Mainwaring, M.C. (2015). Functional properties of nests. In Deeming D.C. and Reynolds S.J, eds. *Nests, Eggs, and Incubation, New Ideas about Avian Reproduction*, 25-49. Oxford University Press, Oxford.

Deeming, D.C. and Pike, T. (2015). Nest surface temperature predicts fledging success of Blue Tits *Cyanistes caeruleus* but not Great Tits *Parus major*. *Acta Ornithologica*, **50**, 247-51.

Deeming, D.C. and Biddle, L.E. (2015). Thermal properties of bird nests depend on air-gaps between the materials. *Acta Ornithologica,* ***50***,121-5.

Deeming, D.C. and Gray, L.A. (2016a). Comparison of two methods for determination of the insulation of passerine nest walls. Avian Biology Research, **9**, 28–31.

Deeming, D.C. and Gray, L.A. (2016b). Incubation attentiveness and nest insulatory values correlate in songbirds. *Avian Biology Research*, **9**,32–6.

East, M.L. and Perrins, C.M. (1988). The effect of nestboxes on breeding populations of birds in broadleaved temperate woodlands. *Ibis*, **130**, 393–401.

Elts, J. (2005). On the nest material of the willow warbler: a quantitative analysis. *Hirundo*, **18**, 31-3.

Ferguson-Lees, J., Castell, R. and Leech, D. (2011). A field guide to monitoring nests. *British Trust for Ornithology*, Thetford.

Gray, L.A. and Deeming, D.C. (2017). Effect of air movement on the thermal insulation of avian nests. *Bird Study*, **4**, 492-501.

Greño, J. L., Belda, E.J. and Barba, E. (2008). Influence of temperatures during the nestling period on post-fledging survival of Great Tit *Parus major* in a Mediterranean habitat. *Journal of Avian Biology*, **39**, 41–9.

Hansell, M.H. (1984). *Animal architecture and building behaviour*. Longman, London.

Hansell, M.H. (2000). *Bird nests and construction behaviour*. Cambridge University Press, Cambridge.

Healy, S.D., Morgan, K.V. and Bailey, I. E. (2015). Nest construction behaviour. DC Deeming and SJ Reynolds, ed. In *Nests*, *Eggs and Incubation*, 16-28. Oxford University Press, Oxford.

Heenan, C.B. (2013). An overview of the factors influencing the morphology and thermal properties of avian nests. *Avian Biology Research*, **6**, 104-18.

Heenan, C.B. and Seymour, R.S. (2011). Structural support, not insulation, is the primary driver for an avian cup-shaped nest design. *Proceedings of the Royal Society B*, **278**, 2924-9.

Heenan, C.B. and Seymour, R.S. (2012). The effect of wind on the rate of heat loss from avian cup-shaped nests. *PLoS ONE*, **7**, e32252.

Hilton, G.M., Hansell, M.H., Ruxton, G.D., Reid, J.M. and Monaghan, P. (2004) Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. *The Auk*, **121**, 777-87.

Honza, M., Øien I.J., Moksnes, A. and Røskaft, E. (1998) Survival of Reed Warbler *Acrocephalus scirpaceus* clutches in relation to nest position. *Bird Study*, **45**, 104-8.

Jelínek, V., Požgayová, M., Honza, M. and Procházka, P. (2016). Nest as an extended phenotype signal of female quality in the great reed warbler. *Journal of Avian Biology*, **47**, 428–37.

Kern, M.D. (1984). Racial differences in nests of White-Crowned Sparrows. *The Condor*, **86**, 455-66.

Lamprecht, I. and Schmolz, E. (2004). Thermal investigations of some bird nests. *Thermochimica Acta*, **415**, 141-8.

Lea, R.W. and Klandorf, H. (2002). The brood patch. In DC Deeming, ed. *Avian incubation: behaviour, environment, and evolution*, 100-18. Oxford University Press, Oxford.

Leniowski, K. and Węgrzyn, E. (2014). Do blackcaps win host-ectoparasite arms race by building lacy nests and shortening nestling period? *Ethology Ecology and Evolution*, **26**, 49-57.

Li, P.J. and Martin, T.E. (1991). Nest-site selection and nesting success of cavity-nesting birds in high elevation forest drainages. *The Auk*, **108**, 405-18.

Li, H., Zhang, M., Yang, X., Cui, L. and Quan., R. (2015). The breeding biology of Red-Whiskered Bulbul (Pycnonotus jocosus) in Xishuangbanna, southwest China. *Zoological Research*, **36**, 233-40.

Lombardo, M.P., Bosman, R.M., Faro, C.A., Houtteman, S.G. and Kluisza, T.S. (1995). Effect of feathers as nest insulation on incubation behavior and reproductive performance of Tree Swallows (*Tachycineta bicolor*). *The Auk*, **112**, 973–81.

Mainwaring M.C. & Hartley I.R. (2008). Seasonal adjustments in nest cup lining in Blue Tits *Cyanistes caeruleus. Ardea*, **96**, 278–82.

Mainwaring, M.C. and Hartley, I.R. (2009). Experimental evidence for state-dependent nest weight in the blue tit, *Cyanistes caeruleus*. *Behavioural Processes*, **81**, 144-6.

Mainwaring, M.C., Hartley, I.R., Bearhop, S., Brulez, K., du Feu, C.R., Murphy, G., Plummer, K.E., Webber, S.L., Reynolds, S.J. and Deeming, D.C. (2012). Latitudinal variation in blue and great tit nest characteristics indicates environmental adjustment. *Journal of Biogeography*, **39**, 1669-77.

Mainwaring, M.C., Hartley, I.R., Lambrechts, M.M. and Deeming, D.C. (2014a). The design and function of birds' nests. *Ecology and Evolution*, **4**, 3909–28

Mainwaring, M.C., Deeming, D.C., Jones, C.I. and Hartley, I.R. (2014b). Adaptive latitudinal variation in Common Blackbird *Turdus merula* nest characteristics. *Ecology and Evolution*, **4**, 841–51.

McGowan, A., Sharp, S.P. and Hatchwell, B.J. (2004). The structure and function of nests of Long-tailed Tits *Aegithalos caudatus*. *Functional Ecology*, **18**, 578–83.

Moksnes, A., Røskaft, E., Hagen L.G., Honza, M., Olsen, C. M. H. 2000. Common Cuckoo *Cuculus canorus* and host behaviour at Reed Warbler *Acrocephalus scirpaceus* nests. International Journal of Avian Science, **2**, 247-258.

Møller, A.P. (1984). On the use of feathers in birds' nests: predictions and tests. *Ornis Scandinavica*, **15**, 38-42.

Moreno, J. (2012). Avian nests and nest-building as signals. *Avian Biology Research*, **5**, 238-51.

Morrison, C.A., Robinson, R.A., Clark, J.A., Leech, D.I. and Gill J.A. (2015). Season-long consequences of shifts in timing of breeding for productivity in Willow Warblers, *Phylloscopus trochilus*. *Bird Study*, **62**, 161-9.

Muth, F. and Healy, S.D. (2011). The role of adult experience in nest building in the zebra finch, *Taeniopygia guttata*. *Animal Behaviour*, **82**, 185-9.

Nord, A. and Williams, J.B. (2015). The energetic costs of incubation. In Deeming, D.C. and Reynolds, S.J., ed. *Nests. Eggs, and Incubation, New Ideas about Avian Reproduction*, 152-70. Oxford University Press, Oxford.

Öberg, M., Arlt, D., Pärt, T., Laugen, T.A., Eggers, S. and Low, M. (2015). Rainfall during parental care reduces reproductive and survival components of fitness in a passerine bird. *Ecology and Evolution*, **5**, 345-56.

Palmgren, M. and Palmgren, P. (1939). Ὺber die wärmeisolierungskapazität verschiedener kleinvogelnester. *Ornis Fennica*, **16**, 1-6.

Pinowski, J., Haman, A,. Jerzak, L., Pinowski, B., Barkowska, B., Grodzki, A. and Haman, K. (2006). The thermal properties of some nests of the Eurasian Tree Sparrow *Passer montanus*. *Journal of Thermal Biology*, **31**, 573-81.

Radford A.N., McCleery, R.H., Woodburn, R.J.W. and M.D. Morecroft. (2001). Activity patterns of parent Great Tits *Parus major* feeding their young during rainfall, *Bird Study*, **48**, 214-20.

Redfern, C.P.F. (2010). Brood-patch development and female body mass in passerines. *Ringing and Migration*, **25**,33-41.

Rodríguez, S. and Barba, E. (2016a). Effects of cool nest microclimates on nestling development: an experimental study with Mediterranean great tits *Parus major*. *Ardeola*, **63**, 251–60.

Rodríguez, S. and Barba, E. (2016b). Nestling growth is impaired by heat stress: an experimental study in a Mediterranean Great Tit population. *Zoological Studies*, **55**, 1-13.

Rohwer, V.G. and Law, J.S.Y. (2010). Geographical variation in nests of yellow warblers breeding in Churchill, Manitoba and Elgin, Ontario. *The Condor*, **112**, 596-604.

Rowley, I. (1990). Behavioural ecology of the Galah. Surrey Beatty & Sons, Chipping Norton, Australia.

Sergio, F., Blas, J., Blanco, G., Tanferna, A., López, L., Lemus, J.A. and Hiraldo, F. (2011). Raptor Nest Decorations are a Reliable Threat Against Conspecifics. *Science*, **331**, 327-9.

Sidis, Y. Zilberman, R. and Ar, A. (1994). Thermal Aspects of Nest Placement in the Orange-Tufted Sunbird (*Nectarinia osea*). *The Auk*, **111**, 1001-5.

Silva, B., Correia, J., Nunes, F., Tavares, P., Varum, H. and Pinto, J. 2010. Bird nest construction – lessons for building with earth. *WSEAS Transactions of Environment and Development,* **6**, 83–92.

Skowron, C. and Kern, M. (1980). The insulation of nests of selected North American songbirds. *The Auk*, **97**,816-24.

Skutch, A. F. 1960. *Life histories of Central American birds II. Pacific Coast avifauna*. 34.

Slagsvold, T. (1989). Experiments on clutch size and nest size in passerine birds. *Oecologia*, **79**, 300-5.

Smith, J.A., Cooper, C.B. and Reynolds S.J. (2015). Advances in techniques to study incubation. In DC Deeming and SJ Reynolds, ed. *Nests, Eggs and Incubation*, *New ideas about avian reproduction*, 179-95. Oxford University Press, Oxford.

Soler, J.J, Ruiz-Castellano, C., Figuerola, J., Martín-Vivaldi, M., Martínez-de la Puente, J., Ruiz-Rodríguez, M. and Tomás, G. (2017). Telomere length and dynamics of spotless starling nestlings depend on nest-building materials used by parents. *Animal Behaviour*, **126**, 89-100.

Storch, D. (1998). Densities and territory sizes of birds in two different lowland communities in eastern Bohemia. *Folia Zoologica*, **47**,181-8.

Snyder, N.F. R., Wiley, J. W. and Kepler, C. B. (1987). *The parrots of Luquillo: Natural history and conservation of the Puerto Rican parrot*. Western Foundation of Vertebrate Zoology, Los Angeles.

Suárez-Rodríguez, M., López-Rull, I. and Macías Garcia, C. (2013). Incorporation of cigarette butts into nests reduces nest ectoparasite load in urban birds: new ingredients for an old recipe? *Biology Letters*, **9**, e20120931.

Surgey, J., du Feu, C.R., and Deeming D.C. (2012). Opportunistic use of a wool-like artificial material as lining of tit (Paridae) nests. *The Condor*, **114**, 1-9.

Taberner Cezero, A. and Deeming, D.C. (2015). Nest insulation in the European Robin (*Erithacus rubecula*): effects of latitude and construction materials. *Avian Biology Research*, **9**, 96-102.

Tiainen, J., Hanski, I. K. and Mehtala, J. (1983). Insulation of nests and the northern limits of three *Phylloscopus* warblers in Finland. *Ornis Scandinavica*, **49**, 149-53.

Tulp, I., Schekkerman, H. and de Leeuw, J. (2012). Eggs in the freezer: energetic consequences of nest site and nest design in Arctic breeding shorebirds. *PLoS ONE*, **7**, e38041.

Turner, J.S. (2002). Maintenance of egg temperature. In DC Deeming, ed. *Avian incubation: behaviour, environment, and evolution*, 119-42. Oxford University Press, Oxford.

Walsh, P.T., Hansell, M.H., Borello, W.D. and Healy S.D. (2010). Repeatability of nest morphology in African weaver birds. *Biology Letters*, **6**, 149-51.

Walsh, P.T., Hansell, M.H., Borello, W.D. & Healy, S.D. (2011). Individuality in nest building: do Southern Masked weaver (*Ploceus velatus*) males vary in their nest-building behaviour? *Behavioural Processes*, **88**, 1–6.

Webb, D. (1987). Thermal tolerance of avian embryos: A review. *The Condor*, **89**, 874–98.

Wesolowski, T. (1996). Natural nest sites of Marsh Tit (*Parus palustris*) in a primeval forest (Białowieża National Park, Poland). *Vogelwarte*, **38**, 235-49.

Wesotowski, T. and Stawarczyk, T. (1991). Survival and population dynamics of Nuthatches *Sitta europaea* breeding in natural cavities in a primeval temperate forest. *Ornis Scandinavica*, **2**,143-54.

Wesolowski, T., Czeszczewik, D., Rowitiski, P. and Walankiewicz, W. (2002). Nest soaking in natural holes- a serious cause of breeding failure? *Ornis Fennica*, **7**, 132-8.

Whittow, G.C. and Berger, A.J. (1977). Heat loss from the nest of the Hawaiian honeycreeper, 'Amakihi'. *The Wilson Bulletin*, **89**, 430-3.

Windsor, R.L., Fegely, J.L. and Ardia, D.R. (2013). The effects of nest size and insulation on thermal properties of tree swallow nests. *Journal of Avian Biology*, **44**, 305-10.

Wysocki, D., Jankowiak, Ł., Greño, J., Cichocka, A., Sondej, I. and Michalska, B. (2015). Factors affecting nest size in a population of Blackbirds *Turdus merula* , *Bird Study*, **62,** 208-16.

Appendix. 1. Individual data for 56 warbler nests used for data collection; location of collection, whether the nest was used for deconstruction for composition materials, used for laboratory "Still air trials", used for "Moving air trials" and the principal collector of hydrological data for each nest.

Nest code	Species	Year	Location	Deconstructed	Laboratory	Wind tunnel	Collection of hydrological properties
BC1	Blackcap	2014	Tyne and Wear	Yes	Yes	Yes	L. Biddle
BC2	Blackcap	2014	Unknown	Yes	Yes	Yes	L. Biddle
BC3	Blackcap	2014	Unknown	Yes	Yes	Yes	L. Biddle
BC4	Blackcap	2014	Unknown	Yes	Yes	Yes	L. Biddle
BC6	Blackcap	2014	Bristol	Yes	Yes	Yes	L. Biddle
2015N10	Blackcap	2015	Unknown	Yes	Yes	Yes	L. Biddle
2015N19	Blackcap	2015	Inverness	Yes	Yes	Yes	L. Biddle
2015N101	Blackcap	2015	Riseholme	Yes	Yes	Yes	L. Biddle
2015N119	Blackcap	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2015N120	Blackcap	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2016N245	Blackcap	2016	Kirkbride	Yes	No	Yes	A. Dickinson
2016N245	Blackcap	2016	Finglandrigg Wood	Yes	No	Yes	A. Dickinson
RW1	Reed Warbler	2014	Spalding	Yes	Yes	Yes	L. Biddle
RW2	Reed Warbler	2014	Spalding	Yes	Yes	Yes	L. Biddle
RW3	Reed Warbler	2014	Spalding	Yes	Yes	Yes	L. Biddle
RW4	Reed Warbler	2014	Spalding	Yes	Yes	Yes	L. Biddle
RW5	Reed Warbler	2014	Spalding	Yes	Yes	Yes	L. Biddle
RW6	Reed Warbler	2014	Spalding	Yes	Yes	Yes	L. Biddle
2014N4	Reed Warbler	2014	Suffolk	Yes	Yes	Yes	L. Biddle
2014N5	Reed Warbler	2014	Suffolk	Yes	Yes	Yes	L. Biddle
2016N24	Reed Warbler	2016	Wicken Fen	Yes	Yes	Yes	A. Dickinson
2016N25	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson

2016N26	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N27	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N28	Reed Warbler	2016	Wicken Fen	Yes	Yes	Yes	A. Dickinson
2016N29	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N30	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N31	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N32	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N33	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N34	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N35	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
2016N36	Reed Warbler	2016	Wicken Fen	No	Yes	Yes	A. Dickinson
WW1	Willow Warbler	2014	Plymouth	Yes	Yes	Yes	L. Biddle
WW5	Willow Warbler	2014	Plymouth	Yes	Yes	Yes	L. Biddle
2015N115	Willow Warbler	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2015N116	Willow Warbler	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2015N117	Willow Warbler	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2015N161	Willow Warbler	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2015N162	Willow Warbler	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2015N163	Willow Warbler	2015	Plymouth	Yes	Yes	Yes	L. Biddle
2016N135	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N136	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N137	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N138	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N139	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N140	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N141	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N142	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N143	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N144	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson

2016N145	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N146	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N147	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N148	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson
2016N149	Willow Warbler	2016	Plymouth	No	Yes	Yes	A. Dickinson